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Contents

Influence of Crop Residue Decay on Soil Nitrates. AUSTIN L. PATRICK.... 335

The Effect of Culture Solutions on Growth and Nitrogen Fractions of Oat Plants at Different Stages of their Development. A. C. SESSIONS AND J. W. SHIVE..... 355

Studies on Nitrogen Absorption from Culture Solutions: I. Oats. A. L. STAHL AND J. W. SHIVE..... 375

Lysimeter Studies: III. The Movement and Translocation of Nitrogen and Organic Constituents in the Profile of a Podzolic Soil. J. S. JOFFE..... 401

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INFLUENCE OF CROP RESIDUE DECAY ON SOIL NITRATES[1]

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Records show that for centuries man has recognized that crops following many legumes resulted in larger yields than when the preceding crops were non-legumes. Farmers in the timothy and red clover sections of the United States realize that the clover sod gives better results than timothy sod. Various reasons have been suggested. Some claim that more organic matter is added by the clover sod and appear to think that this accounts for the beneficial effect. A common belief is that the clover sod supplies large amounts of nitrogen. Others claim that the deeper root system of the clover brings mineral plant food constituents to the surface.

During the past few years agronomists have begun to attribute the greater immediate value of clover residue over that of timothy and other non-legumes in part at least to the effect on the availability of the nitrogen.

The present work was undertaken to throw some additional light on the perplexing problems of why crops following red clover should yield more than those following timothy or corn. In carrying out this study time did not permit an investigation of all of the factors which might be responsible for the different residual effects of these crops. In fact, attention was concentrated on whether the amounts of clover, timothy, and corn residues left in the field after harvest were sufficient to effect the disappearance or accumulation of nitrate nitrogen. This necessitated obtaining an approximate idea of the amounts of these crop residues (roots and stubble) left in the field at harvest. After this information was obtained it was planned to use these same percentages of residues to soil under controlled conditions in the laboratory in order to determine the effect on the nitrate content of the soil.

The literature dealing with root development of field crops is extensive. Weaver (38) and Miller (23) have made fairly complete bibliographies. Most of the work has been done in the short and tall grass soil sections of the United States. Here the lower soil horizons, according to Marbut (21), are not unlike the surface soils in texture and in structure. Consequently, King (14), Hays (6, 7), Ten Eycke (29, 30), and Weaver (38) have found extensive root development of many cultivated crops below depths of 2 feet.

The soils in the humid timbered soil section of eastern United States as a group are charac-

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[1] Thesis submitted in partial fulfillment of the requirement for the degree of doctor of philosophy at Cornell University.

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terized by shallow surface and compact heavy lower horizons. McCool (20) states that there is a marked difference both in weight and in length of roots on different soil types. Carlson (2) noticed that in compact soils all strains and varieties of alfalfa developed branch roots. Laird (15) found that the percentage of Bermuda grass roots found in the upper 8 inches of soil increased very markedly as the clay content of a sandy soil was increased.

The work of Goff and Beckwith (5) and Jones (13) in New York, Hickman (9) in Pennsylvania, Newman (24) in Alabama, and Woods (43) in Connecticut tends to prove that the main development of roots of nearly all cultivated plants in the eastern part of the country are in the upper 3 to 10 inches of soil.

The disappearance of all or a portion of the nitrates from the soil following the incorporation of crop residues of high carbon and low nitrogen content has been shown by a number of investigations. The literature on this subject has been thoroughly reviewed by Collison and Conn (3), Lyon (16), and Waksman (33) to 1927. Rahn (25) states that the addition of large amounts of energy-rich material causes an increase in the number of soil microörganisms. The microörganisms utilize the soluble nitrogen and convert it into protein. Rahn's explanation seems to be the one accepted by the majority of those who have worked on this subject. Lyon and Bizzell (18), Lyon, Bizzell, and Wilson (19), Wilson and Wilson (41), Holtz and Singleton (10), and Martin (22) have tested the effect of crop roots on nitrification in one way or another and agree that such materials do effect the accumulation or disappearance of soil nitrates.

Collison and Conn (3) believe that in addition to the disappearance of nitrates there is a toxic chemical agent which acts immediately upon plants after germination following applications of straw and other plant residues. This toxic idea has been dispersed pretty well by the work of Viljoen and Fred (32) and Blair and Prince (1).

Studies made by Waksman and Tenny (35, 36, 37), Waksman and Skinner (34), Whiting and Richmond (40), Wilson and Wilson (42), Jensen (12), Heck (8), and Rege (26), tend to show that the carbon-nitrogen ratio alone of materials does not tell the whole story regarding the relative effect on nitrification when incorporated in the soil.

Two bulletins published in 1930 tend to emphasize the practical importance of the subject under consideration. Lyon (17) and Collison and Mensching (4) give figures that indicate that legume roots and stubble stimulate the yield, whereas timothy residues depress the yield of crops that follow.

EXPERIMENTAL

The present experiment was designed to determine whether the roots and stubble of red clover, maize, and timothy are present at harvest in sufficient amounts to cause a marked change in the nitrate content of a soil.

The percentage of the residues (roots and stubble) in the soil for each of the crops was first determined, then the effect of field amounts of residues on nitrification was determined in the laboratory under controlled conditions, in three series of experiments. In the first series the field amounts of clover were taken as the basis of the amount of materials to be added. Similar amounts of nitrogen were applied in the form of timothy and maize residues. The second series was based on the amount of timothy residues (roots and stubble) found in the field. Here the effects of the two residues were compared when equal carbon amounts of each were added. In the third series a comparison of the effect of the two residues was made, where the carbon-nitrogen ratios of the two were made equal by the addition of nitrate of soda to the tumblers of soil receiving timothy residues.

Another set-up consisted of determining the effect of applying the water-soluble constituents from the residues of each of the three crops.

In addition to the determination of nitrates, total bacteria counts were made and the carbon dioxide evolved was measured. A test for vanillin in the residues of the crops used was made. General plant analyses of different kinds were also included in the experiment.

A preliminary study[3] consisted of checking at definite periods the change in soil nitrates caused by the addition of varying amounts of corn roots to soil, starting with a quantity too small to have any material effect upon the nitrates of the soil, then using a number of increasingly larger amounts until a change was produced. Similar amounts of nitrogen were added to soil in other tumblers in the form of dried blood. This afforded a comparison between the effects of additions of materials with widely varying carbon-nitrogen ratios.

The surface soil of the Hagerstown silt loam taken from virgin land lying adjacent to the experimental orchard on the farm of the Pennsylvania State College was used in this experiment. Mechanical, chemical, and mineralogical analyses of soil from this same place have been reported by Thomas (31).

In obtaining this soil the top inch of sod was discarded. The soil immediately below was screened through a 2-mm. screen into large galvanized iron cans, which were kept tightly closed. Since this experiment covered a long period, soil was taken on several different occasions. When it was too moist to be screened in the field it was spread in a thin layer on oilcloth in a building until it was sufficiently dry. At no time was thoroughly air-dried soil used. Before any experiment was set up the moisture content of the soil was always determined.

The lime requirement of the soil according to the Truog method showed it to be nearly neutral. The pH of the soil was slightly above 7.5. The addition of 0.3 gm. of calcium carbonate to 100 gm. of soil failed to affect the reaction according to pH determinations.

Methods of determining weights of roots and stubble

The method used in collecting the root and stubble data on the timothy and red clover fields was a modification of several methods. Laird (15) recently reported using a somewhat similar method. Galvanized iron cylinders, 8 inches deep and 8 inches in diameter, reinforced with a heavy iron hoop at the top were driven about 7 inches into the soil at representative spots in the field, the tops of the plants having first been cut so as to leave about 2 inches of stubble.

Next the full cylinders were dug out of the soil, weighed, and placed in closely fitting wire baskets (8 meshes to the linear inch), which were then placed in a tub of water. In a short time the cylinders were easily pulled off, leaving the soil columns in the baskets.

[3] This preliminary study was used as a portion of a thesis for a master of science degree at the Pennsylvania State College; since it has a direct bearing on this problem and has never been published, it is presented as part of this paper.

The baskets of soil were set in 2-mm. soil screens, many short pieces of wire were pushed through them from one side to the other. The stubble in each basket was attached by means of a cord to some of the top wires. These precautions prevented the mass of roots and stubble from falling into the bottom of the basket. The soil was washed away with a gentle stream of water. After the crop residues were removed from the baskets, they were spread between blotters to dry over night. The next day after foreign organic matter had been removed they were placed in paper bags until thoroughly air dried. Moisture determinations were made on the soil samples collected for this purpose when the cylinders were dug from the soil. The average weight of the soil in the first 11 cylinders weighed was used as the weight of the soil cylinders thereafter.

When the weight of dry matter in roots and stubble in a cylinder of soil and the weight of the soil and the percentage of water in it were known, the percentage of dry matter in the soil was easily figured. The weight of the residue per acre was figured directly, using the area of the top of the cylinder. The subsoil root weights of clover were obtained in two places by the method just described. The cylinder driven into the subsoil was a small iron one 6 inches in diameter and 6 inches deep. The chances of error in obtaining weight of subsoil roots seemed greater than in the surface soil.

An approximate idea of the amount of maize roots and stubble was obtained by digging a trench about 3 feet deep, cutting a row of maize hills through the middle. The surface soil was washed with a hose from the portion of the roots that remained in the soil. The weights thus obtained were multiplied by 2 to give the weight per hill. The weight per acre was obtained by multiplying the weight per hill by the number of hills in an acre. The acre weight of soil used in obtaining per cent figures was 2,000,000 pounds.

Air-dried roots and stubble of each of the three crops were ground in a Wiley mill and stored in stoppered containers for use in connection with the problem.

Preparation of soil cultures

The containers used for the nitrification studies were ordinary tumblers with tin pill boxes serving as covers. In case bacteria counts were to be made in addition to nitrates determined, 110 gm. of soil was used, otherwise only 100 gm.

Calcium carbonate (0.3 gm. per 100 gm. of soil) and finely ground dry organic materials were mixed with the soil prior to wetting. Sufficient distilled water was added to bring the total moisture content to 20 per cent (dry basis). An additional 5 ml. of water was used for each gram of crop residues applied. Where nitrate of soda or the extracts from plant residues were used they were applied in the water. The tumblers were kept in drawers in the laboratory and water was added from time to time as needed. The temperature during incubation averaged about 20°C.

The moist soil was placed in standard 2.5-liter acid bottles for making the carbon dioxide determinations. Each rubber stopper was fitted with two glass

tubes, the outer ends of which were kept closed by means of rubber tubing and pinch cocks. Except for the type of container and the amount of soil used, the preparations for the carbon dioxide and the nitrification studies were similar.

Methods of chemical analyses

The total nitrogen was determined by the official Gunning method; pumice stone was used instead of zinc dust. Nitrates were determined by the method of Schreiner and Failyer (27). The experiments were so set up that at each determination period three tumblers of soil for each treatment were used. The soil was mixed in battery jars with 500 ml. of water per 100 gm. of soil. Calcium oxide was used to help clarify the solution before filtering. In series 2 and 3 the soil was placed in percolation tubes with cotton plugs and water was added until 500 ml. had come through. In this case a saturated solution of lime water was used before clear water was added. The latter method was simpler than the former and seemed equally satisfactory. The total carbon analysis was made by the chromic acid method as perfected by White ahd Holben (39).

In series 1 the carbon dioxide was collected in soda lime by means of an absorption train similar to the one described by White and Holben in the total carbon analysis. In series 2 and 3 the carbon dioxide was collected in an excess of approximately 0.1*N* barium hydroxide, the excess being determined by titrating with 0.1*N* hydrochloric acid, phenolphthalein being used as an indicator. The apparatus for this purpose was designed by Merkle.[4]

In testing water extracts from plant residues for vanillin the resorcin test used by Collison and Conn (3) was employed.

The general plant analyses were made as described by Waksman and Tenny (36), 4 gm. of plant material being used instead of 2. Half of the cold-water soluble material was used for determining the soluble nitrogen and the other was taken down to dryness for soluble organic matter determination. The percentage of lignin, ash, and protein-free analysis was made according to the Schwalbe (28) method, duplicate samples being used to determine the nitrogen content. The hydrogen-ion determinations were made with a potentiometer.

Amounts of crop residues in soils

The figures showing the weights of the roots and stubble of the surface soil were obtained on the farms of the College of Agriculture at Cornell University. The soil was the Dunkirk silty clay loam as described by Howe, Buckman, and Lewis (11). The data were obtained early in July, 1926. In the opinion of those in charge of the farm operations this was an average season for the growth of timothy and clover. The timothy field was part of the Caldwell Experimental Field and had been fairly heavily fertilized. This was the second year timothy crop.

Table 1 shows that under the conditions described the average amount of timothy residue in the surface soil is over twice as great as the residue of clover.

[4] F. G. Merkle, associate professor of soil technology, Pennsylvania State College.

The same is true if we compare the largest number of pounds of residue of both crops or the smallest of each. It is of interest to note in this connection that the timothy root figures reported by Jones (13) agree with these fairly well. The cylinders used in getting the red clover data extended down to the heavy compact subsoil. The taproot of clover tapered rapidly and where it entered

TABLE 1

Amount of red clover and timothy roots and stubble in the surface soil of the Dunkirk silty clay loam

SAMPLE NUMBER	RED CLOVER ROOTS AND STUBBLE WATER-FREE BASIS		TIMOTHY ROOTS AND STUBBLE WATER-FREE BASIS	
	Pounds per acre	Per cent in the soil	Pounds per acre	Per cent in the soil
1	9,544	0.50	11,810	0.63
2	8,233	0.43	9,893	0.53
3	7,710	0.43	10,867	0.58
4	5,527	0.31	11,148	0.59
5	5,046	0.26	8,508	0.45
6	5,635	0.28	13,053	0.69
7	4,891	0.27	19,493	1.04
8	5,284	0.28	16,338	0.87
9	7,156	0.38	14,881	0.77
10	4,665	0.25	11,511	0.61
11	3,903	0.21	12,915	0.69
12	.....	....	11,406	0.61
Average.....	6,144	0.33	12,652	0.69

TABLE 2

Amount of maize residue left in the surface soil of the Hagerstown silt loam. Moisture-free basis

HILL NUMBER	PER CENT RESIDUE IN SOIL	POUNDS PER ACRE
1	0.053	1,052
2	0.059	1,182
3	0.057	1,133
4	0.044	885
5	0.041	829
6	0.04	805
7	0.048	961
Mean	0.049	978

the subsoil layer it split into two fine twine-like branches. The portion by weight of the plant that was found in the 6–12-inch layer of soil is comparatively unimportant: the weight of red clover roots was 134.7 pounds per acre; of this, 0.008 per cent and 0.0077 per cent, respectively, were found in this layer in two samples. A number of very fine thread-like timothy roots entered the subsoil layer. Their total weight was estimated to be very small.

In the light of this information we could not attribute greater crop yields following red clover than following timothy to greater amounts of organic matter added by the clover.

The data (table 2) pertaining to maize residue (roots and stubble) were obtained on the Hagerstown silt loam soil on September 25, 1926. This soil is underlain by a heavy clay or clay loam subsoil, which, in the place where the maize roots were collected, came within 7 inches of the surface. It was noticed here that only a very few fine maize roots entered the heavy layer. This point was also mentioned by Hickman (9) who worked on this same soil in 1887.

The maize, which was one of the tall growing varieties of dent, was just ripe. A fair crop of maize was obtained in central Pennsylvania that season.

TABLE 3

Analysis of residues and dried blood

MATERIAL	PER CENT MOISTURE-FREE BASIS		CARBON-NITROGEN RATIO
	Nitrogen	Carbon	
Clover residue	1.6707	38.4166	22.9:1
Maize	0.607	35.0965	57.8:1
Timothy	0.5257	42.4921	80.8:1
Dried blood	10.787	45.35	4.2:1

TABLE 4

Amounts of materials added in series 1

MATERIAL	LOW	MEDIUM	HIGH
	gm.	*gm.*	*gm.*
Clover residue	0.2	0.33	0.5
Maize residue	0.5502	0.9079	1.374
Timothy residue	0.634	1.0461	1.585

Series 1

Nitrification of residues. The nitrification studies in series 1 were based on the amounts of clover residues found in the field. The three amounts of this material used approximated the low, average, and high amounts found. In making up the maize and timothy residue soil mixtures the amounts added supplied the same quantity of nitrogen as were supplied by the clover residues. Table 3 shows the analysis of these residues and dried blood as to the total carbon and nitrogen.

The exact amounts of the different materials used per 100 gm. of soil on a moisture-free basis is shown in table 4.

The amounts of maize residue and the high amount of timothy materials added are higher than the amounts found in the field. The low amount of the timothy residue used is about the same as the average amount found in the

field, and the medium amount added corresponds very closely to the largest amount found in the field.

Table 5 shows that field amounts of clover residue caused a very slight depression in soil nitrates during the first period of incubation. Even this depression was broken by periods of slight accumulation of nitrates. All amounts of timothy residues used had a very pronounced depressing effect on soil nitrates; not a single determination showed an increase of nitrates over the corresponding no treatment. It is evident that where the amounts of tim-

TABLE 5

Variation of nitrates (NO_3) from no treatment

Expressed as parts per million of dry soil

INCUBATION PERIOD	TREATMENT PER 100 GM. OF DRY SOIL					
	Equal amounts of nitrogen		Equal amounts of nitrogen		Equal amounts of nitrogen	
	2 gm. of clover residues	Timothy residues	0.33 gm. of clover residues	Timothy residues	0.5 gm. of clover residues	Timothy residues
days						
1	−1.3	−11.0	−.8	−9	−4	−9
2	+1.9	−7.6	+2.2	−7	+20	−4
4	−.8	−20.6	−2.6	−20	−9	−19
7	−.9	−60.6	−11	−68	−27	−62
10	+16.7	−39.5	+29.4	−60	+3	−48
14	−24.1	−76.2	−5.2	−80	−6	−60
21	+19.8	−134.6	+9	−156	−28	−139
28	−46.7	−137.9	−34	−142	−26	−142
35	−56.3	−186	−24	−196†	−13	−136
42	−53	−130.8	−23	−173†	−10	−173*
49	−44.0	−200	−24	−231	+45	−236
56	−52.7	−229	−36	−273	+11	−289
70	−205.6	−266	−204	−355	−68	−381*
84	−14.7	−131	+11	−150	+10	−230
105	−7.3	−100	+18	−151	+35	−245
135	+4.0	−123	+26	−130	+24	−213
210	+43	−33	+120	−34	+34	−128

* All nitrates disappeared.

othy roots and stubble approach the amounts used in this experiment a prolonged depression of nitrates will follow. The larger the amounts of timothy residues added the longer was the depression period severe. In general the depression was very marked from the second to the tenth week. In the case of the two larger additions of timothy residues there was an entire disappearance of nitrates at times. The rather irregular fluctuation of the nitrate curves may be due in part to temperature variations in the laboratory.

The additions of maize roots and stubble corresponding to the three clover residue applications affected soil nitrates as shown in table 6.

There was a rather marked nitrate depression below the corresponding no treatment. As in the case of timothy the larger the amounts of material added the more lasting seems to be the severe depression period. In the case of the small application the depression was not continuous nor very marked except in the seventh-day determination.

None of the amounts of maize residues found in the field amounted to more than 0.06 per cent in the surface soil. The preliminary study (table 7) shows the effect of the addition of seven different amounts of maize roots on the soil nitrates. The smallest amount applied was 0.2 gm. to 100 gm. of dry soil. This amount appears not to have caused any nitrate depression during the first 12 weeks. The larger amounts of roots up to 1 gm. caused a slight nitrate depression which lasted from 6 to 12 weeks. When amounts of more than 1 gm. were used the depression was more noticeable. In this work stubble was not

TABLE 6

Effect on soil nitrates of adding varying amounts of maize residue

Results expressed as a variation of nitrates in parts per million of dry soil, from no treatment.

INCUBATION PERIOD	TREATMENT PER 100 GM. OF DRY SOIL		
	0.5502 gm.	0.9079 gm.	1.374 gm.
days			
1	14	30	30
2	6	−45	−70
4	65	85	55
7	−148	−135	−117
10	−64	−59	−266
14	−17	−43	−126
21	7	−75	−207
28	−14	−14	−81
42	28	−57	−147

mixed with the roots. The addition of a material with a very narrow carbon-nitrogen ratio like dried blood (table 8) tended to increase nitrate accumulation.

Since the percentage of roots and stubble in the field is so very low it looks as though maize does not cause any marked change in soil nitrates in the field. In the immediate vicinity of large clumps of roots and stubble this statement would not hold.

Carbon dioxide accumulation. The amounts of carbon dioxide evolved from soils treated with the various residues were determined over a period of 28 days. In preparing the cultures for this work residues were added on an equal nitrogen basis. Only one amount of each residue was used. The low clover residue amount (0.2 gm. per 100 parts of soil) was taken as the standard.

Immediately after the experiment was set up large amounts of carbon dioxide were evolved (fig. 1). This was especially noticeable in the case of the timothy

residue treatment but was not so pronounced in the check soil. After the fourth day the differences per period for any one treatment were not so marked. Toward the end of the period all of the curves show a tendency to flatten out. This is less true of the timothy treatment curve than of the others. The carbon dioxide curve of the clover residue treatment never exceeded, or even approached, the treatment of the timothy residues. This would indicate that there was no sudden falling off of the activities of microörganisms, as one would

TABLE 7

Effect of varying amounts of maize roots (no stubble) on the accumulation of nitrates in the soil

NUMBER	TREATMENT PER 100 GM. OF DRY SOIL	NITRATES EXPRESSED IN PARTS PER MILLION OF DRY SOIL AFTER				
		2 weeks	6 weeks	12 weeks	18 weeks	24 weeks
1	No treatment	90.21	123.11	231.06	364.16	300
2	0.2 gm. of corn roots	90.33	141.57	245.33	282.44	268
3	0.4 gm. of corn roots	65.35	112	182.50	389.33	324.5
4	0.6 gm. of corn roots	61.30	120.2	219.16	417	291.83
5	0.8 gm. of corn roots	46.82	98.5	187	396	262.66
6	1.0 gm. of corn roots	37.76	107.50	179.33	376.33	282.16
7	2.0 gm. of corn roots	23.41	39.81	315.83	366	280.16
8	3.0 gm. of corn roots	5.98	11.03	200.16	402.66	238.66

TABLE 8

*Effect of dried blood on the accumulation of nitrates in the soil**

NUMBER	NITRATES EXPRESSED IN PARTS PER MILLION OF DRY SOIL AFTER				
	2 weeks	6 weeks	12 weeks	18 weeks	24 weeks
1	90.21	123.11	231.06	364.16	300
2	69.55	180.33	212.5	474.51	310.66
3	92.25	198.44	301.16	475.66	271.83
4	97.57	230.22	323.33	528.66	208.16
5	100.23	273.08	418.83	573.83	332.66
6	110.66	335.5	444.50	643.50	386.5
7	107.83	440.28	732.33	736.5	883.33
8	128.21	574.09	765.6	978.5	1127.33

* Treatment added dried blood to furnish same amounts of N as the corresponding treatments of corn roots in table 7.

infer from the bacteria counts. It would point to the conclusion that organisms other than bacteria shown on the plates were active.[5] These must be important in assimilating soluble nitrogen. In fact these "other" organisms must have been largely responsible for the major portion of the evolution of carbon dioxide and accumulation of nitrogen after the first few days.

[5] Total counts of bacteria by the plate method were included in this study. These results have been omitted in order to save space.

The figures show that the maize residue treatment evolved less carbon dioxide up to and including the tenth day than did the clover residues. The difference between these two materials is further shown from the different effects on nitrate depression. Where nearly equal amounts of the two materials are used the immediate depression is greater from clover residue treatments. This is rather surprising since the carbon-nitrogen ratio of maize is considerably wider than that of the clover. That there is a difference in availability of nitrogen or energy material or both is apparent. Differences in carbon-nitrogen ratios of different material do not appear to tell the entire story.

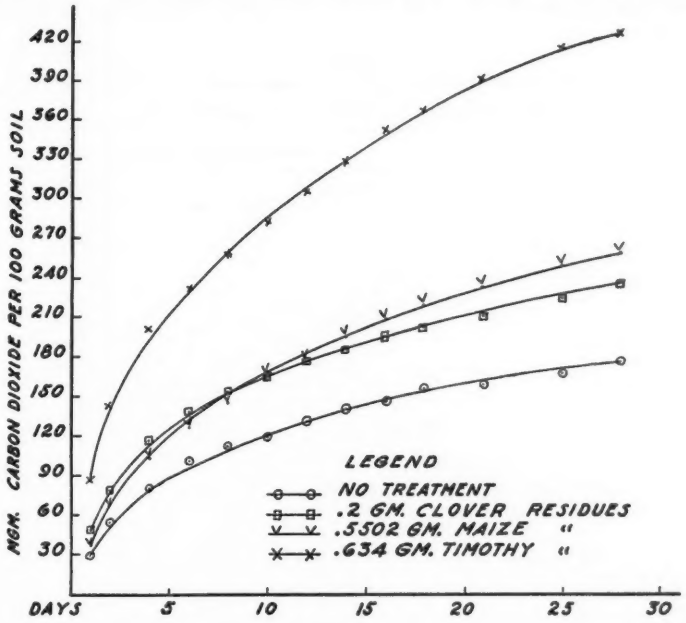

FIG. 1. TOTAL AMOUNTS OF Co_2 EVOLVED FROM SOIL TREATED WITH EQUAL QUANTITIES OF NITROGEN IN DIFFERENT CROP RESIDUES

Nitrification of water-soluble extracts of residue. Equal weights of timothy and clover residues, ground to pass a coarse screen (2 mm.) were soaked in distilled water (30 parts of water to 1 of residue) for 2 days. At the termination of this time the extracts were filtered and applied to soil in tumblers. Each 100 gm. of soil received the leachings from 0.4 gm. of residue.

The results of these studies are shown in table 9. Both extracts caused a depression of nitrates. The depression was more marked in the treatment receiving timothy extract than in the clover extract treatment.

Qualitative tests for vanillin in the extracts from the residues of clover,

maize, and timothy failed to show the presence of this toxic material. Collison and Conn (4) found this and other toxic organic compounds in extracts from straw. They were of the opinion that the immediate ill results that often followed applications of straw were due in part to a chemical toxic substance.

Series 2

The purpose of this study was to compare the effects of the additions of clover and timothy residues on soil nitrate changes, when they were applied on an equal carbon basis. The basic figure used for this study was 0.4 gm. of timothy residue per 100 gm. of soil. This amount was chosen since it was a little below

TABLE 9

Soil nitrate changes induced by additions of water extracts from crop residues

Results expressed as variation from untreated soil

INCUBATION PERIOD	NITRATES IN P.P.M. DRY SOIL	
	Timothy	Clover
days		
1	−4.1	1.9
2	4.6	2.0
4	−9.2	9.6
7	−57.0	−14.4
10	−15.8	−11.2
14	−43.8	−5.2
21	6.5	45.6
28	−57.4	−30.0
35	−44.7	−3.0
42	−36.0	−3.3
49	−14.0	−29
56	−26.0	19
70	.....	.....
84	−29.3	4.0
105	−37.0	1
133	−24.0	−14.0
210	60.0	66.0

the lowest amount of this residue found in the field. The same amount of carbon was supplied by 0.45 as by 0.4 gm. of timothy. Figure 2 shows the results of this work. The clover residues caused just as marked a depression of nitrates during the first 18 days as did the timothy. After 18 days, nitrates began to accumulate in the clover residue treatments more rapidly than in the other treatment. From shortly after the twenty-seventh day until the end of the experiment there was very little difference in nitrate contents between the untreated and clover residue treated soils. The timothy residues continued to show a depression of nitrates during the entire 60 days.

A study of the rates and total amounts of carbon dioxide evolved from soils

treated as in the nitrate work was made. The total evolution of carbon dioxide was much greater from the clover treatments than from the timothy (table 10). The greatest difference between the two treatments occurred within the first two weeks. The latter part of the sixty day run found the timothy treatment evolving only slightly more carbon dioxide than the clover.

It is interesting to note that the greatest depression of nitrates in the case of the clover residue treatment occurred at the time of the greatest carbon dioxide

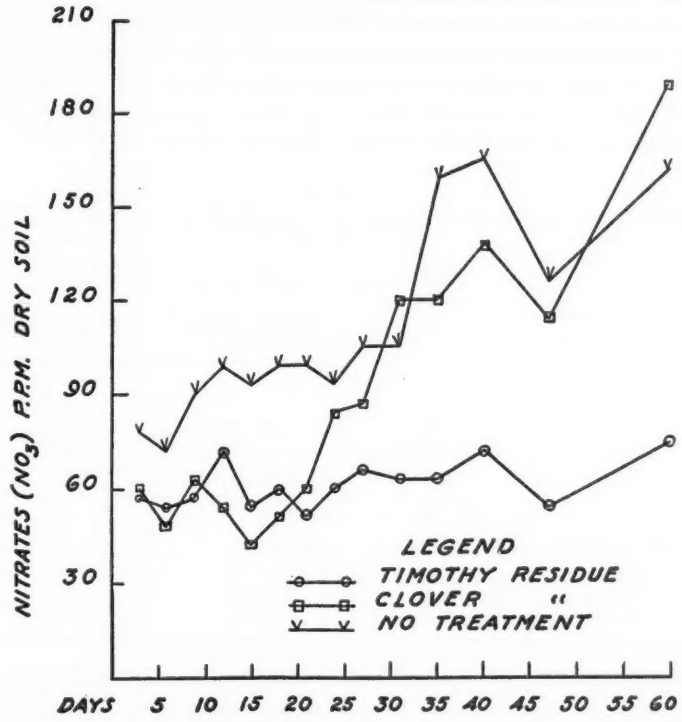

FIG. 2. EFFECT OF PLANT RESIDUES ON CHANGES IN SOIL NITRATES WHEN ADDED IN EQUAL CARBON AMOUNTS

evolution. There are no pronounced breaks in either the carbon dioxide or the nitrate curves of the timothy treatment.

The two materials having been applied on an equal carbon basis the changes in nitrates and carbon dioxide evolution must be due to factors other thon differences in total organic carbon contents. The total analyses show that there is a much narrower carbon-nitrogen ratio in the case of the clover residues than in the timothy residue. It would appear then that the lack of nitrogen prevented a more rapid evolution of carbon dioxide from the timothy residue.

This is another way of saying that biological activity was limited in the case of the timothy residue by lack of nitrogen. Heck (8) states:

> A given amount of energy material when used by soil organisms requires a given amount of nitrogen for decomposition and this nitrogen is built up into organic form in the microbial substance. Any nitrogen in excess of this amount is liberated in the mineral form.

Series 3

The carbon-nitrogen ratios of clover and timothy residues were made the same in another set of soil cultures. This was done by adding sufficient nitrogen in the form of nitrate of soda to the timothy residue in the series 2 treatment. A check treatment consisting of soil plus a similar quantity of nitrate of soda was set up at the same time. The effect of this treatment on nitrate depression and carbon dioxide evolution is given in figure 3 and table 11.

TABLE 10

Amounts of carbon dioxide evolved from soils treated with timothy and clover residues applied on an equal carbon basis

ASPIRATION PERIODS	MGM. CARBON DIOXIDE PER 100 GM. OF SOIL (MOISTURE-FREE)		
	Check (no treatment)	Variation from check due to addition of timothy residues	Variation from check due to addition of clover residues
days			
3-day periods			
0–3	6.572	16.583	22.578
3–6	8.772	13.475	26.648
6–9	9.306	12.172	25.184
9–12	10.26	12.10	14.22
4-day periods			
12–16	11.33	9.74	12.10
16–20	10.56	12.82	11.97
20–24	8.8	10.51	11.23

The addition of sodium nitrate to the soil alone did not materially effect the biological activities of the soil. At least the rate and amounts of carbon dioxide evolved from the soil with sodium nitrate and the one without were very nearly the same. The addition of nitrogen caused an increase in the evolution of carbon dioxide when added to the timothy treatment. A comparison of the carbon dioxide accumulation of the clover residue treatment with that of the nitrate of soda timothy residue treatment show the two sets of figures to be very similar. The clover treatment evolved more total carbon dioxide than did the timothy plus sodium nitrate treatment. The greater difference came within the first 2 weeks. After the first 2 weeks the two treatments produced about the same amounts of carbon dioxide for some time. The last few weeks of the 60-day experiment, the rate of evolution of carbon dioxide from the timothy plus nitrogen exceeded that of the clover treatment.

Timothy residues plus sodium nitrate caused a very marked disappearance of nitrates over soil plus sodium nitrate. In comparison to the clover treatment the timothy plus sodium nitrate at all times contained more nitrates than did the clover treatment.

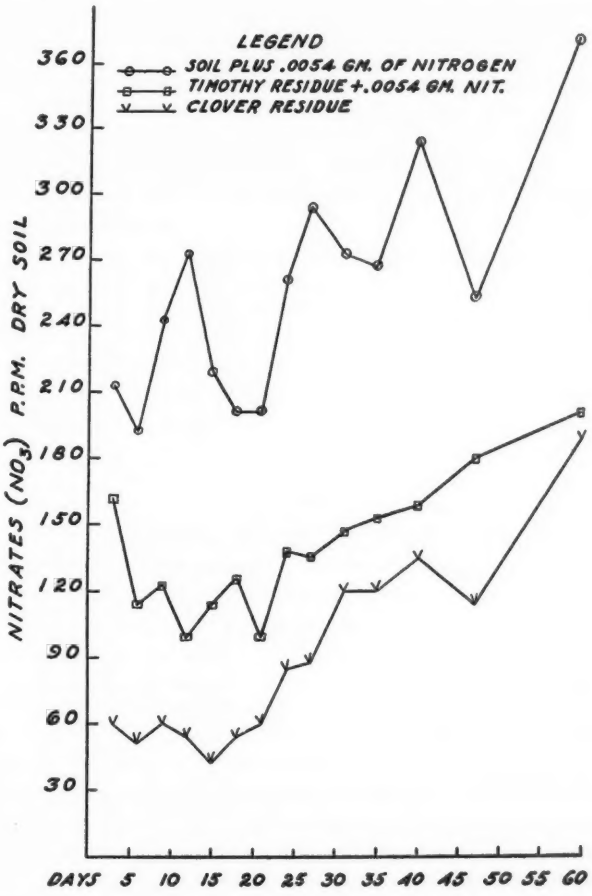

FIG. 3. EFFECT OF ADDITION OF PLANT RESIDUES ON ACCUMULATION OF SOIL NITRATES

The applications of residues were made on an equal carbon basis. Nitrate of soda was added to the timothy residue in amounts sufficient to make the C/N ratios of the treatments the same.

From a review of the results of both types of experiments used in series 2 and series 3 it is evident that there is a very marked difference in nitrates and in carbon dioxide evolved from soils treated with equal carbon amounts of

timothy and clover residues. These differences are not so noticeable where the carbon nitrogen ratios of the two materials have been made the same by addition of sodium nitrate to the timothy treatment.

TABLE 11

Amounts of carbon dioxide evolved from soil treated with timothy and clover residues

Carbon-nitrogen ratios were made equal by addition of sodium nitrate with the timothy residue.

ASPIRATION PERIODS	MGM. OF CARBON DIOXIDE EVOLVED PER 100 GM. DRY SOIL		
	Check (nitrate of soda only)	Variations from check due to additions of timothy residue and nitrate of soda	Variation from check due to addition of clover residues
days			
3-day periods			
0–3	5.475	20.432	23.675
3–6	8.882	21.89	26.538
6–9	11.99	15.18	22.50
9–12	8.25	17.88	16.23
4-day periods			
12–16	10.45	13.95	12.98
16–20	9.27	14.21	13.26
20–24	10.93	8.59	8.10

TABLE 12

General analysis of roots and stubble of timothy, clover, and corn on a moisture-free basis

	PER CENT IN 4 GM. OF FINELY GROUND MATERIAL		
	Timothy residue	Clover residues	Corn residues
Ether extract	0.35	0.71	0.79
Organic matter soluble in cold water	23.51	17.83	8.67
Lignin (ash-free, protein free)	32.11	35.32	38.41
Protein insoluble in cold water	2.32	7.00	1.54
Ash	6.89	13.54	19.23*
Cellulose and pentosans†	34.82	26.30	31.36
Total	100.00	100.00	100.00
Soluble nitrogen	.154	.545	.265
Part of total nitrogen soluble	30.3	34.8	43.65

* The high ash content must be due in part to adhering particles of soil which could not be washed off.

† Obtained by difference.

General analyses of residues

General analyses of clover, corn, and timothy residues were made. The methods were similar to those used by Waksman and Tenny (36). The results

of these analyses (table 12) show many differences between the three residues which might be responsible for the differences observed in the evolution of carbon dioxide and nitrate changes when these three materials were applied to the soil.

Among the many differences shown by these analyses perhaps the most striking and important are the figures which show the cold-water soluble nitrogen and organic matter. Wilson and Wilson (42) worked with roots of sorghum and maize on an equal weight basis. They noticed a greater immediate depression of nitrates following an application of sorghum roots than following maize roots. They attributed this difference to the greater amount of soluble energy applied by the sorghum roots. Whiting and Richmond (40) believe that the largest single factor in the initial rate of decomposition is the amount of water-soluble nitrogen.

It would seem that the immediate effect on the change in nitrate concentration in the soil solution upon the addition of residues can best be explained upon differences of both soluble nitrogen and soluble organic matter. The C : N ratios of the water-soluble portions of these residues are approximately; corn 5:1, clover 10:1, and timothy 88:1.

That water extracts from the two residues (clover and timothy) cause a difference in nitrate depression has been shown. The water extract applied to each 100 gm. of soil was obtained from 0.4 gm. of residue. In series 2 the amount of timothy residue applied was 0.4 gm. It is possible therefore to compare the effect of the extract and the material in this case. After the first 2 weeks the nitrate depression caused by the timothy residue added directly was more severe than that caused by the timothy extract. Up to the first 2 weeks there seemed to be little difference between the two treatments. A considerable proportion of the nitrate depression certainly seems to be caused by the soluble organic material. The later depression must be attributed in part to the insoluble constituents.

The literature review brought out the fact that cellulose and other relatively insoluble organic compounds caused a depression of soil nitrates. Waksman and Tenny (37) and Rege (26) have shown that lignin is a fairly resistant material, which does not decompose rapidly in the soil. Waksman and Tenny base this conclusion on the fact that in the "so-called soil humus and humic acid" portion of the soil, lignin occupies a leading rôle. They designate the organic matter remaining after subtracting the sum of the lignin and ether-soluble extracts from the total organic matter as "available energy material." The analyses of the residues show that timothy contains the largest proportion of available energy material from this viewpoint. Rege's work shows that the presence of a large percentage of lignin is detrimental to the decomposition of cellulosic matter. It seems to act as an inhibiting factor; perhaps it acts as a physical barrier.

SUMMARY

A study was made to determine whether the amounts of roots and stubble of red clover, maize, and timothy present at harvest time are sufficient to cause a marked change in the nitrate content of soil.

The amounts of roots and stubble left in the soil, at harvest time, were determined. The effects of additions of various amounts of crop residues to soil were determined both upon changes in soil nitrate and upon the evolution of carbon dioxide. General plant analyses were also made.

The amount of dry matter in the soil in the form of red clover roots and stubble amounts to only about half the weight of timothy roots and stubble from equally good stands. The percentage of dry matter in the soil in the form of corn roots and stubble is exceedingly small in comparison with that of the other two crops.

The actual weight of roots of these crops found below 7 inches was relatively unimportant. The soils from which these crops were taken are characterized by fairly heavy subsoils, which probably accounts for the meager root development in the lower horizons.

The nitrification studies show that field amounts of clover and timothy are sufficient to influence the accumulation of soil nitrates. All amounts of timothy residues used resulted in a depressing effect of nitrates over the no treatment. The larger the amounts of timothy residues added, the longer was the depression period severe. Clover residues seemed to cause a temporary depression of soil nitrates during the first period of incubation.

Nitrification studies where corn residues were added to the soil showed that before soil nitrates were affected by addition of this material a much larger amount had to be added than is found in the field except in the immediate vicinity of large clumps of roots and stubble.

Carbon dioxide curves show that high carbon dioxide evolution is correlated rather closely with nitrate depression.

Water extracts from timothy residues, produced, when mixed with soil, a greater depression of soil nitrates than did another extract from clover residues.

The carbon-nitrogen ratios of clover and timothy residues were made the same in another set of soil cultures. This was done by adding the residues on an equal carbon basis and supplementing the timothy treated cultures with nitrogen in the form of sodium nitrate.

Timothy residues plus sodium nitrate caused a very marked disappearance of nitrates over soil plus sodium nitrate. In comparison to the clover treatment the timothy plus sodium nitrate at all times contained more nitrates. The addition of sodium nitrate to the soil did not produce any marked change in carbon dioxide evolved. The carbon dioxide evolution was greatly increased by the addition of sodium nitrate to the cultures receiving the timothy residues.

General analyses show a number of interesting facts. Timothy residues which caused the greatest depression of soil nitrates contained the highest per-

centage of organic matter soluble in cold water. The timothy residues also contained the largest amount of so-called available energy material and the least amount of water-soluble nitrogen of the three residues. Corn, which caused the least depression of soil nitrates, contained the smallest percentage of organic matter soluble in cold water and the least amount of available energy material. It did not contain the highest percentage of water-soluble nitrogen, but the carbon-nitrogen ratio of the water-soluble portions was narrowest in corn of the three residues.

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THE EFFECT OF CULTURE SOLUTIONS ON GROWTH AND NITROGEN FRACTIONS OF OAT PLANTS AT DIFFERENT STAGES OF THEIR DEVELOPMENT[1]

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In recent years culture solutions have been commonly employed in studying the nutritional needs of economic plants and the influence of various elements upon plant growth. A knowledge of the chemical nature of the media in which plants are grown, such as is furnished by these synthetic cultures, has been of primary value in the solution of many nutritional problems.

Many investigations have been carried out which deal with the influence of the several elements required by plants in major quantities, their proportions, and concentrations in culture media, upon growth and yields. It is now generally conceded that the range of salt proportions in a culture solution favorable to plant growth is relatively wide. Hoagland (4) states "there is not sufficient evidence that plants require for optimum yields any very specific ratio of elements within wide limits, providing the total supply and concentration of the essential elements are adequate." But, under the usual cultural methods, total supply is noticeably limited, and concentrations of some of the essential elements change rapidly unless some adequate system of continuous flow for the constant renewal of these elements is employed. It is, however, possible, as will be brought out and as has previously been suggested, that plants of different ages may require different proportions of the essential elements for optimum growth.

That the composition of plants varies with the composition of the solution in which they are grown, has been known since the days of de Saussure (7). Hoagland (4), Dickson (1), and others (2, 3) have shown that with an increase of an element in the solution there is a corresponding increase of that element within the plant; but how the more complex organic materials such as the carbohydrates, fats, and proteins are affected by the ionic relations of the solution in which the plants are grown, has not as yet been thoroughly investigated.

The primary purpose of the present work was to investigate the quantitative relationships between the essential inorganic constituents of a series of culture solutions of known composition and the nitrogenous nature and quantity of

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plant tissue which these solutions are capable of producing. It involves a quantitative study of various nitrogen fractions of oat plants and how these fractions are influenced by the salt proportions, particularly with respect to the proportions of the nitrogen bearing salts, of the solutions in which they are grown, at different stages of development throughout the life cycle.

PLAN OF EXPERIMENTS

Three series of oat plants, designated as A, B, and C, were grown in culture solutions. Each series included 20 individual cultures, and all solutions differed in their salt proportions in accordance with a definite plan. The corresponding solutions of the three series were identical, and all cultures were similarly treated to every respect except that the plants from the three series were harvested when 20, 40, and 60 days old, respectively.

At the time of harvest the plants of series A were in the early seedling stage, having an average height of 28 cm. Those of series B were in the early vegetative stage and heads were just beginning to form in the sheaths. The height of the plants at this harvest varied somewhat, but averaged about 50 cm. When the plants of series C were harvested they were well past the heading stage. The size of the plants at this age varied greatly, depending upon the salt proportions of the solutions in which they were grown. Some plants in the poorly balanced solutions failed to produce panicles.

The plants were grown in the greenhouse during the summer months—June, July, and August. The temperature averaged about 25°C. and there was an average daylight period of approximately 15 hours.

Description of solutions

The 20 culture solutions employed were chosen from the Tottingham complete series of 84, and were modified as described by Jones and Shive (5). This modification consisted of the substitution of $(NH_4)_2SO_4$ for KNO_3 in equivalent partial osmotic concentrations, and each solution when complete possessed a total osmotic concentration of one atmosphere. The solutions chosen were uniformly distributed throughout the Tottingham series and will be designated by the culture numbers referring to the position which they occupy in the series and on the four-coördinate diagrammatic scheme employed by Tottingham, as well as by the values of their numerical succession from 1 to 20. The solution cultures were prepared from half-molecular stock solutions of the salts employed. The partial volume-molecular concentrations of the salts as they occurred in the different solutions, the solution numbers indicating their position in the modified Tottingham series (5) are given in table 1.

In order to avoid any pronounced change in either concentration or salt proportions of the solutions through the action of the growing plants, the solutions were continuously renewed at constant rates by the method described by Shive and Stahl (10). Two and one-half liters of fresh solution were passed through each culture jar during a 24-hour interval.

Iron was added to the cultures in the form of ferrous sulfate. A fresh 0.1 per cent solution was prepared each day and added in amounts which varied according to the needs of the plants. An average of about 1 cc. of this solution per liter of culture solution was employed. Much less than this was use during the seedling stage and proportionately more during the vegetative and reproductive phases.

Cultures and material employed

Seven and one-half liter glazed stone crocks were used as culture receptacles. A tightly fitting circular lid was made for each crock from beaver-board and

TABLE 1

Description of culture solutions

SOLUTION NUMBER	CULTURE SOLUTION DESIGNATION	PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			
		KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	$MgSO_4$
1	$T_1R_1C_1$	0.00211	0.0014	0.00146	0.01659
2	$T_1R_1C_3$	0.00211	0.0014	0.00438	0.01185
3	$T_1R_1C_5$	0.00211	0.0014	0.00730	0.00711
4	$T_1R_1C_7$	0.00211	0.0014	0.01022	0.00237
5	$T_1R_3C_1$	0.00211	0.0042	0.00146	0.01185
6	$T_1R_3C_3$	0.00211	0.0042	0.00438	0.00711
7	$T_1R_3C_5$	0.00211	0.0042	0.00730	0.00237
8	$T_1R_5C_1$	0.00211	0.0070	0.00146	0.00711
9	$T_1R_5C_3$	0.00211	0.0070	0.00438	0.00237
10	$T_1R_7C_1$	0.00211	0.0098	0.00146	0.00237
11	$T_3R_1C_1$	0.00633	0.0014	0.00146	0.01185
12	$T_3R_1C_3$	0.00633	0.0014	0.00438	0.00711
13	$T_3R_1C_5$	0.00633	0.0014	0.00730	0.00237
14	$T_3R_3C_1$	0.00633	0.0042	0.00146	0.00711
15	$T_3R_3C_3$	0.00633	0.0042	0.00438	0.00237
16	$T_3R_5C_1$	0.00633	0.0070	0.00146	0.00237
17	$T_5R_1C_1$	0.01065	0.0014	0.00146	0.00711
18	$T_5R_1C_3$	0.01065	0.0014	0.00438	0.00237
19	$T_5R_3C_1$	0.01065	0.0042	0.00146	0.00237
20	$T_7R_1C_1$	0.01477	0.0014	0.00146	0.00237

heavy waxed paper. The heavy paper was cut into strips 5 cm. wide and sufficiently long to encircle the crock. The strip was folded longitudinally so that the folded halves stood at a right angle to each other. Successive cuts were then made at intervals of about 1.5 cm. from one outer edge to the center of the horizontal strip so as to allow the cut edges to overlap each other when the strip was brought into a circular form around the crock. The cut side was then sewed firmly to the lower surface of the circular board lid in such a manner that the uncut side formed a flange around the cover which served to hold the cover firmly in position on the crock. Six holes, each 5 cm. in diameter, were bored in the lid and a tightly fitting cork stopper was placed in each hole. The entire lid was then dipped in melted paraffin.

A pure line of oats (Burt Nebraska 4) was used. Healthy seeds of the same general size were chosen and germinated between blotting paper. When the primary roots were 3 mm. long, selection of similar seedlings was made and they were transferred to a germinating net such as was used by Shive (9). The root tips were inserted in a solution having an osmotic concentration of 0.5 atmosphere and the following salt porportions: KH_2PO_4, 0.00211; $(NH_4)_2SO_4$, 0.00420; $Ca(NO_3)_2$, 0.00730; and $MgSO_4$, 0.00237 moles per liter. After a few days the seedlings were carefully selected for uniformity as to height (5–6 cm.) and general appearance, placed in the cork stoppers according to the method employed by Tottingham (11), and transferred to the culture jars. In series B and C 3 plants were placed in each cork making a total of 18 plants per culture, but in order to obtain sufficient green tissue for analysis for series A, the plants of which were harvested when only 3 weeks old, it was necessary to place 6 plants in each cork stopper making a total of 36 plants to each culture.

ANALYTICAL PROCEDURE

Handling of tissue. Only the tops of the oat plants were used. They were severed from the roots and lower stems 2.5 cm. above the cork stopper and about 3.5 cm. above the uppermost root. As the plants from each culture were harvested their green weight was immediately determined. The tissue was then rapidly cut into sections about $\frac{1}{2}$ mm. long and these were thoroughly mixed. From this minced material duplicate samples were taken, accurately weighed, and dried for moisture determinations, while the bulk of the tissue was immediately weighed and used for aqueous extractions of the nitrogen fractions.

Extraction and determination of the nitrogen fractions. In obtaining the plant extract, the fresh tissue was ground in a large mortar with nitrogen-free quartz sand. Water was added to this material and the whole was transferred to a large funnel over which was placed an 18-inch square of lawn cloth. The extract was forced through the cloth by moderate wringing by hand. The resulting extract was heated to boiling, 3 cc. of 10 per cent acetic acid was added, and boiling was continued for 3 or 4 minutes. The coagulum formed was removed from the solution by filtering through a Büchner funnel into which were placed a filter paper and some nitrogen-free paper pulp. The coagulum-free filtrate was reduced in volume to 500 cc. by gently boiling, and 100-cc. aliquots were taken for analysis. It was found that by this procedure the non-coagulable extracted nitrogen was completely removed, and additional grinding and further extraction gave no increase of nitrogen in this extract.

Ammonia and nitrate nitrogen. These determinations were made on 100-cc. aliquots of the coagulum-free extract, using a method (8) by means of which the two fractions, ammonia and nitrate nitrogen, can be determined on the same sample with a high degree of accuracy. Any nitrite nitrogen present is included as nitrate nitrogen.

Inorganic nitrogen. This fraction is obtained by adding together the nitrogen from the ammonia and nitrate determinations.

Soluble organic nitrogen. Since all forms of inorganic nitrogen found in plant tissues are considered to be soluble, the soluble organic nitrogen was obtained by subtracting the total inorganic from the total soluble nitrogen.

Insoluble organic nitrogen. This fraction is often designated as "protein nitrogen," but since it is obtained by subtracting the soluble nitrogen from the total nitrogen, it includes not only the protein nitrogen but the insoluble nitrogen from other substances, such as glucosides, nitrogenous lipoids, plant bases, and nucleic acids.

Total organic nitrogen. This includes all the organic nitrogen forms, soluble and insoluble, and is obtained by subtracting the total inorganic nitrogen from the total nitrogen of the plant.

Total nitrogen. This was determined by Kjeldahlization of some of the tissue previously used for moisture determinations. The determinations were made according to the directions given by Ranker (6), care being taken to adhere to his suggestions in every detail.

EXPERIMENTAL RESULTS

Growth relations and dry weights

The dry weight of the plants produced by the individual cultures was determined in the usual way. Some interesting relationships exist between the dry weights of the plants at the different stages of development and the chemical composition of the solutions in which they were grown, as will be brought out in the consideration of the yields from the three series of plants here described.

The numerical data representing the yield values for each of the three series are presented in table 2, together with the relative osmotic proportions of the salts in the various solutions. For the sake of convenience in comparing the effect on yields shown by differences in the osmotic proportions of the nitrogen bearing salts in the culture solutions, it will be observed that the cultures and the data obtained from them are grouped in pairs. The grouping is such that, except for the last three pairs, the osmotic values of $MgSO_4$ in the two solutions of each pair are the same, as are also the osmotic values of KH_2PO_4. It will be observed, however, that while the sums of the osmotic concentrations from the two introgen bearing salts are equal in the two solutions of each pair, the concentration of the ammonium salt in the first solution of each pair is low and the nitrate salt is high, while in the second solution of each pair the ammonium salt is correspondingly high and the nitrate salt correspondingly low. Thus, in the third pair of cultures (numbers 18 and 19), $MgSO_4$ has an osmotic value of one-tenth of the total osmotic concentration of one atmosphere in each solution, and KH_2PO_4 in each solution has an osmotic value of five-tenths of the total osmotic concentration of the solution. The osmotic concentration of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ in the first solution of the pair is one-tenth and three-tenths and in the second solution three-tenths and one tenth, respectively,

of the total osmotic concentration of the solution. In the last three pairs of cultures in the table, the nitrogen bearing salts show the same osmotic values in each pair. This arrangement greatly facilitates comparisons with respect to the effect on yields of the differences in the osmotic values of the two nitrogen bearing salts. All the succeeding tables are constructed in a similar

TABLE 2

Influence of ammonium and nitrate nitrogen in the culture solutions on the growth of oat plants of different ages

PAIRS OF CULTURES	SOLUTION NUMBER		OSMOTIC PROPORTIONS				DRY WEIGHT OF 20 PLANTS		
			$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A, plants 20 days old	Series B, plants 40 days old	Series C, plants 60 days old
							gm.	*gm.*	*gm.*
1	12	$T_3R_1C_3$	3	3	1	3	**1.70**	**11.80**	**25.75**
	14	$T_3R_3C_1$	3	3	3	1	**1.45**	5.80	8.83
2	2	$T_1R_1C_3$	5	1	1	3	1.22	**10.56**	24.06
	5	$T_1R_3C_1$	5	1	3	1	1.15	3.90	8.37
3	18	$T_5R_1C_3$	1	5	1	3	1.14	**10.55**	**24.22**
	19	$T_5R_3C_1$	1	5	3	1	1.27	10.24	14.15
4	3	$T_1R_1C_5$	3	1	1	5	1.20	9.31	**26.50**
	8	$T_1R_5C_1$	3	1	5	1	1.29	5.72	8.08
5	13	$T_3R_1C_5$	1	3	1	5	1.39	10.07	16.72
	16	$T_3R_5C_1$	1	3	5	1	1.21	8.55	11.22
6	4	$T_1R_1C_7$	1	1	1	7	1.21	7.34	**24.58**
	10	$T_1R_7C_1$	1	1	7	1	1.30	6.17	Killed
7	7	$T_1R_3C_5$	1	1	3	5	**1.42**	9.34	17.20
	9	$T_1R_5C_3$	1	1	5	3	**1.54**	9.00	14.62
8	20	$T_7R_1C_1$	1	7	1	1	1.28	7.40	20.01
	1	$T_1R_1C_1$	7	1	1	1	0.80	4.59	7.42
9	17	$T_5R_1C_1$	3	5	1	1	1.13	8.12	22.71
	11	$T_3R_1C_1$	5	3	1	1	1.23	5.77	8.86
10	15	$T_3R_3C_3$	1	3	3	3	**1.70**	**10.56**	**27.80**
	6	$T_1R_3C_3$	3	1	3	3	1.18	**10.66**	23.90

manner. The dry weights of the cultures producing the highest five yields in each series appear in bold-face type.

From an examination of the yield values of the younger and older plants as presented in table 2, it will be observed that solutions favorable to good growth of the younger plants (series A) do not always correspond with those producing

most favorable growth in the older plants of series B and C. The five solutions producing high yields in the series of young plants (series A) are numbers 7, 9, 12, 14, and 15; whereas in the series of the older plants (series C) they are solutions number 3, 4, 12, 15, and 18. The pronounced differences in yields between solution 20 and 1, and also 17 and 11 of pairs 8 and 9 respectively, in series B and C may not be due so much to the nitrogen bearing salts, which are very low in these cultures, as to the general unbalanced condition of the salt proportions in these cultures.

Thus when the plants are in the early stages of development, there is no very pronounced difference between the yields from the high ammonia and high nitrate cultures. In four out of the seven pairs of cultures in which the nitrogen bearing salts show differences in their osmotic values, the larger yields for series A are slightly in favor of the high ammonia cultures. This condition, however, is strongly reversed in the older plants (series B and C). In every case the solutions relatively high in ammonium nitrogen and low in nitrate nitrogen greatly retarded the growth of the older plants, whereas the high yields were produced by the cultures relatively low in ammonium nitrogen and relatively high in nitrate nitrogen. This clearly brings out the fact that from the standpoint of dry weight production the young plants are just as efficient in the utilization of ammonium nitrogen as they are in the use of nitrate nitrogen, whereas the older plants utilize nitrate nitrogen much more effectively than they do ammonium nitrogen. Some interesting relations between these yield values and the nitrogenous composition of the plants from the various solutions will be presented.

It is clear from the data of table 2 that in the utilization of nitrogen as ammonium, the young plants are much more efficient than are the older plants, whereas in the use of nitrate nitrogen the older plants are much more efficient than the young plants, when measured by the criterion of dry plant material produced.

Presentation of analytical data

Nitrogen as ammonia. The numerical data representing the results of the analysis for the inorganic nigrogen fractions in the plants of series A (20 days), B (40 days), and C (60 days) are presented in table 3. The data are given in percentages of nitrogen as ammonia and as nitrate on the dry weight basis. The cultures, together with the corresponding analytical data, are arranged in pairs in the same order as in table 2.

Although the nitrogen as ammonia in the plants of the three series is low and represents but a small fraction of the total inorganic nitrogen in the plants, yet this fraction increases or decreases, but not in direct proportion, with increase or decrease respectively of nitrogen as ammonium in the solutions in which the plants were grown. It will be observed that three cultures high in nitrogen as ammonia in each series are corresponding cultures. All the high yielding cultures in each of the three series are associated with a high relative

proportion of ammonium sulfate in the solutions in which the plants were grown, and this relation is the same in the young plants as it is in those of intermediate age and in the mature plants. On the other hand, low yields of nitrogen as ammonia are always associated with low relative proportions of

TABLE 3

Ammonia nitrogen, nitrate nitrogen, and total inorganic nitrogen in plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBER	OSMOTIC PROPORTIONS				NITROGEN AS AMMONIA			NITROGEN AS NITRATES			INORGANIC NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C	Series A	Series B	Series C	Series A	Series B	Series C
						per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	12	3	3	1	3	0.06	0.04	0.06	1.45	1.34	0.93	1.51	1.38	0.99
	14	3	3	3	1	0.09	0.10	**0.35**	1.73	0.95	1.20	1.82	1.05	**1.55**
2	2	5	1	1	3	0.06	0.04	0.06	1.87	**1.51**	1.21	1.93	**1.55**	1.27
	5	5	1	3	1	0.06	**0.15**	0.21	1.75	0.95	0.92	1.81	1.10	1.13
3	18	1	5	1	3	0.07	0.06	0.08	1.93	**1.80**	1.33	2.00	**1.86**	1.41
	19	1	5	3	1		0.11	**0.22**		1.31	1.26		1.42	1.48
4	3	3	1	1	5	0.05	0.06	0.06	**2.32**	**1.72**	1.25	**2.37**	**1.78**	1.31
	8	3	1	5	1	**0.14**	**0.17**	**0.40**	1.61	1.05	1.18	1.75	1.22	**1.56**
5	13	1	3	1	5	0.09	0.04	0.08	1.89	1.35	**1.35**	1.98	1.30	1.43
	16	1	3	5	1	**0.15**	**0.17**	**0.55**	1.73	1.06	0.68	1.89	1.22	1.23
6	4	1	1	1	7	0.06	0.05	0.06	**2.16**	**1.94**	**1.37**	**2.22**	**1.99**	1.43
	10	1	1	7	1	**0.20**	**0.32**	Killed	1.55	0.91	Killed	1.75	1.23	
7	7	1	1	3	5	0.09	0.08	0.15	**2.08**	**1.64**	**1.66**	**2.17**	**1.72**	**1.81**
	9	1	1	5	3	**0.13**	**0.18**	**0.28**	1.80	1.30	**1.74**	1.93	1.48	**2.02**
8	20	1	7	1	1	0.06	0.06	0.07	1.69	1.23	1.02	1.75	1.29	1.09
	1	7	1	1	1	0.07	0.07	0.08	1.57	1.07	1.06	1.64	1.14	1.14
9	17	3	5	1	1	0.06	0.06	0.07	1.77	1.42	0.96	1.83	1.48	1.03
	11	5	3	1	1	0.08	0.06	0.11	1.61	1.06	1.07	1.69	1.12	1.18
10	15	1	3	3	3	**0.10**	0.08	0.14	**2.03**	1.40	1.13	**2.13**	1.48	1.27
	6	3	1	3	3	0.09	0.08	0.11	**2.16**	1.31	**1.41**	2.25	1.39	**1.52**

ammonium sulfate in the solutions in which the plants were grown, regardless of the age of the plants.

To bring out the relation between nitrogen as ammonia in the plants of the various cultures and the age of the plants; that is, the effect of age on the accumulation of nitrogen as ammonia in the plants, the data of table 3 repre-

senting this fraction in series A were arranged in the descending order of their values. These data were plotted to form the graph of figure 1. The ordinates represent the percentage of nitrogen as ammonia, and the abscissas represent the different cultures of the series arranged in the descending order of the ammonia nitrogen values of the plants of series A. The corresponding data for the mature plants (series C) were then plotted, using the same abscissas and the same scale of ordinates.

From the graphs of figure 1, it is clear that although the percentage of nitrogen as ammonia is low throughout both series, the values for series C are always higher than are the corresponding values for series A. Although many values for corresponding cultures of the two series show only slight differences, several corresponding cultures of the two series show differences which are quite pronounced: as, for example, culture 16 ($T_3R_5C_1$) and culture 8 ($T_1R_5C_1$), or the values of the first cultures represented by the graph, in which the solu-

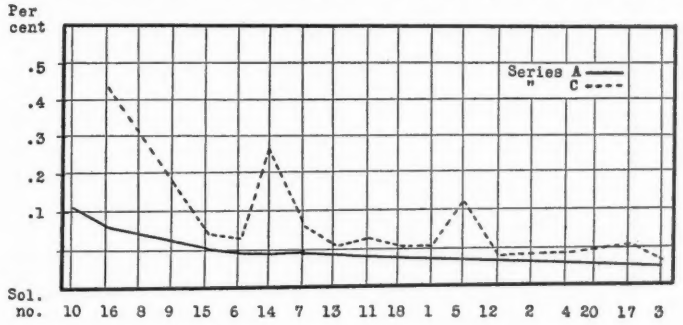

FIG. 1. GRAPHS SHOWING THE DIFFERENCE IN PERCENTAGES OF NITROGEN AS AMMONIA IN THE PLANTS OF CORRESPONDING CULTURES OF SERIES A (AT 20 DAYS) AND SERIES C (AT 60 DAYS)

tions were relatively high in ammonium sulfate, and also in cultures 14 ($T_3R_3C_1$) and 5 ($T_1R_5C_1$) in which a high proportion of the nitrogen in these solutions was present in the form of the ammonium salt.

It is to be noted as a matter of considerable interest, that nitrogen as ammonia is the only nitrogen fraction here considered which exhibits higher values for the older plants than for the younger plants of corresponding cultures. This accumulation of nitrogen as ammonia as the plants become older produced marked injurious effects in the plants grown in solutions with high proportions of ammonium sulfate, as has already been cearly brought out in connection with the discussion of dry weight yields. In every case, an increase of nitrogen as ammonia within the plants in the course of their development, as indicated in table 3, is directly correlated with decreased yields of dry plant material, as shown in table 2.

It appears that during the early stages of growth the plants are capable of

assimilating ammonium nitrogen as rapidly as they absorb it, even from solutions with relatively high concentrations of this form of nitrogen. As the plants become older, this power to assimilate ammonium nitrogen appears to diminish, under the conditions of these experiments, resulting in accumulation of ammonium nitrogen in the tissues, retardation of growth, and in some instances actual injury to the plants.

Nitrogen as nitrates. It will be observed from the data of table 3 that nitrate nitrogen comprises by far the greater portion of the inorganic nitrogen of these plants, and that the nitrate content of the plants of a given series varies with, but not in proportion to, the nitrate content of the solution in which the plants were grown. In general, cultures which produced plants high in nitrate nitrogen, regardless of age, are associated with high relative proportions of calcium

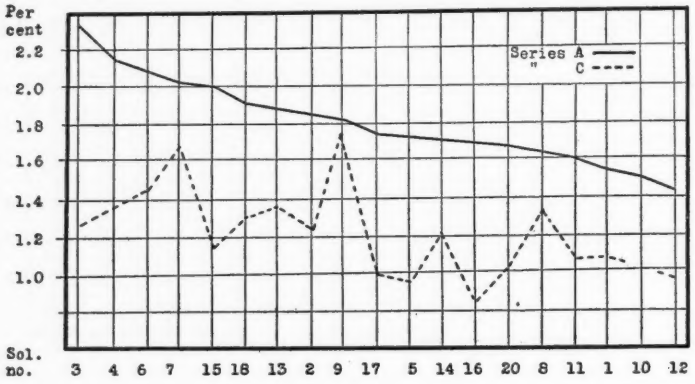

FIG. 2. GRAPHS SHOWING THE DIFFERENCES IN PERCENTAGES OF NITROGEN AS NITRATE IN THE PLANTS OF CORRESPONDING CULTURES OF SERIES A (AT 20 DAYS) AND SERIES C (AT 60 DAYS)

nitrate in the solutions in which the plants were grown; but this relation does not hold in all cases, but it is particularly pronounced in the cultures of series B.

Although the variation in the nitrate content of plants of the same age, resulting from differences in the chemical nature of the solutions with respect to nitrogen content, is not particularly pronounced except in series B, the variation in the nitrate content of plants of corresponding cultures at different ages is quite striking. The young plants of series A always show a higher nitrate content than do the older plants of series B and series C. This is clearly brought out by the graphs of figure 2. The data referring to nitrogen in the nitrate form found in the plants of series A as given in table 3 were arranged in the descending order of their values and plotted to form the upper graph of the figure. The corresponding values for nitrogen as nitrates in the plants of series C were then plotted in the same order, using the same scale of ordinates. The values of series B lie intermediate between those of series A and series C

and are not here shown. These graphs show clearly the marked differences in the nitrate values of the old and young plants of corresponding cultures. The marked decrease in this fraction as the plants increase in age is interesting, since, as previously shown, the ammonia nitrogen representing the other inorganic nitrogen fraction increased as the plants became older. The average

TABLE 4

Soluble organic nitrogen in plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBER	OSMOTIC PROPORTIONS				SOLUBLE ORGANIC NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C
						per cent	*per cent*	*per cent*
1	12	3	3	1	3	1.03	0.43	1.06
	14	3	3	3	1	0.76	0.10	**1.30**
2	2	5	1	1	3	0.71	0.63	0.71
	5	5	1	3	1	0.49	0.42	1.06
3	18	1	5	1	3	0.98	0.49	0.70
	19	1	5	3	1		**0.74**	0.95
4	3	3	1	1	5	0.89	0.60	0.71
	8	3	1	5	1	**1.22**	**0.96**	**1.27**
5	13	1	3	1	5	1.03	0.38	0.68
	16	1	3	5	1	**1.16**	0.60	**1.97**
6	4	1	1	1	7	1.02	0.53	0.89
	10	1	1	7	1	**1.27**	**1.38**	Killed
7	7	1	1	3	5	1.10	**0.75**	1.06
	9	1	1	5	3	**1.27**	**1.11**	**1.60**
8	20	1	7	1	1	0.90		0.58
	1	7	1	1	1	0.82	0.02	0.60
9	17	3	5	1	1	1.01	0.40	0.67
	11	5	3	1	1	0.96	0.20	0.56
10	15	1	3	3	3	**1.21**	0.65	**1.09**
	6	3	1	3	3	0.93	0.44	0.79

proportion of ammonia in the plants of series C is 79.3 per cent higher than the average in the plants of series A; while at the same time the average nitrate nitrogen in the plants of series C is 34.8 per cent less than this average in the plants of series A.

Soluble organic nitrogen. This fraction refers to the organic nitrogen found in the coagulum-free extract of the plants. Just what these soluble organic

nitrogenous compounds are is not known, but they are thought of as diffusible organic forms and building blocks for the more complex or conjugated protein. The percentage of nitrogen found in this form represents but a small fraction of the total organic nitrogen of the plants.

The data pertaining to the soluble organic nitrogen fractions for series A, B, and C are presented in table 4 in the same manner as were the data of previous tables. An examination of the data of this table brings out the fact that a relatively high content of soluble organic nitrogen in the plants is always associated with high proportions of nitrogen as ammonium in the solutions producing these plants, and that low yields of soluble organic nitrogen in each of the three series are definitely associated with relatively low proportions of nitrogen as ammonium in the culture solutions, regardless of the age of the plants. On the other hand, the data show that there is no such relation between the soluble organic nitrogen content of the plants and the nitrate nitrogen or the solutions in which they were grown.

A comparison of the data of table 3, relating to the ammonium nitrogen fraction, with those of table 4, representing the soluble organic nitrogen fraction of corresponding cultures of the three series, shows that cultures which produced high yields or low yields of one of these two fractions produced high yields or low yields, respectively, of the other fraction also.

As already pointed out, high and low values of these two fractions are definitely correlated with high and low proportions, respectively, of nitrogen as ammonium in the culture solutions. To emphasize these relations and to bring out more clearly the pronounced influence of the ammonium salt in the solutions upon the soluble organic nitrogen fraction and upon the content of nitrogen as ammonia in the plants, the values of these two fractions for the plants of series A, B, and C have been brought together and are diagrammatically presented in figure 3. On the left of the figure are given the percentage values of nitrogen as ammonia in the plants, and on the right the precentage values of the water-soluble organic nitrogen. At the base of the figure are represented in diagram the osmotic compositions of the nitrogen bearing salts, below which are given for each solution the osmotic values of the four salts in tenths of the total osmotic concentration of one atmosphere. The pairs of cultures are numbered to correspond with those given in the tables representing the values of the various nitrogen fractions.

These diagrams representing the three series of cultures show remarkable agreement between the nitrogen as ammonia and the total soluble organic nitrogen of the plants here considered. They bring out the fact that the values of these two nitrogen fractions of the plants are determined in large measure by the nitrogen as ammonium in the culture solutions: that is, in general, high proportions of nitrogen as ammonium in the culture solutions correspond to high yields of both soluble organic nitrogen and nitrogen as ammonia in the plants. With but one or two exceptions in series A and B, this relation is exact. On the other hand, the opposite relation exists with respect to these

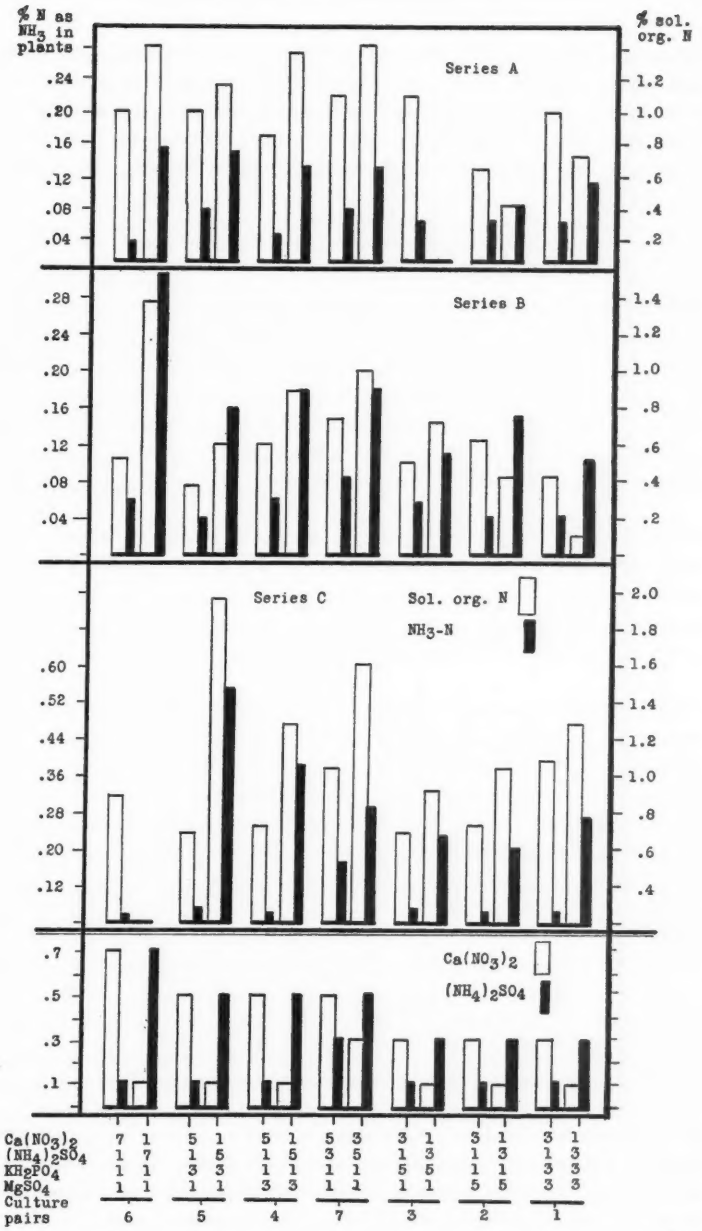

FIG. 3. DIAGRAM SHOWING THE RELATION BETWEEN THE OSMOTIC PROPORTIONS OF THE NITROGEN BEARING SALTS OF THE CULTURE SOLUTIONS AND THE SOLUBLE ORGANIC AND THE AMMONIA NITROGEN IN THE PLANTS

two nitrogen fractions and the nitrate nitrogen of the solutions: high yields of these two fractions in the plants correspond to low proportions of nitrate nitrogen in the culture solutions, and low yields correspond to high proportions.

Insoluble organic nitrogen. This fraction represents the nitrogen found in the protoplasm, or the higher products of nitrogen synthesis. The numerical

TABLE 5

Insoluble organic nitrogen plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBERS	OSMOTIC PROPORTIONS				INSOLUBLE ORGANIC NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C
						per cent	*per cent*	*per cent*
1	12	3	3	1	3	3.17	2.77	1.63
	14	3	3	3	1	3.69	3.12	1.34
2	2	5	1	1	3	**3.83**	2.60	**2.21**
	5	5	1	3	1	**4.14**	**3.39**	**2.28**
3	18	1	5	1	3	3.14	3.04	1.70
	19	1	5	3	1	Lost	2.88	1.68
4	3	3	1	1	5	3.39	2.53	2.19
	8	3	1	5	1	**3.83**	3.27	2.05
5	13	1	3	1	5	3.62	2.85	1.77
	16	1	3	5	1	**3.85**	2.98	**2.32**
6	4	1	1	1	7	3.69	**3.50**	2.14
	10	1	1	7	1	**3.98**	2.84	Lost
7	7	1	1	3	5	3.81	**3.33**	1.98
	9	1	1	5	3	3.73	2.75	**2.26**
8	20	1	7	1	1	2.53	Lost	1.65
	1	7	1	1	1	3.33	**3.31**	2.09
9	17	3	5	1	1	3.62	2.91	1.69
	11	5	3	1	1	3.23	**3.48**	1.64
10	16	1	3	3	3	3.60	3.21	2.13
	6	3	1	3	3	3.80	3.12	**2.45**

data representing this fraction are given in table 5. As might be expected, for plants of a given age, these vital portions of the living cells are less influenced by the type of solutions in which the plants are grown than are the intermediate or lower forms of nitrogen compounds. There appears to be no definite relation between the insoluble organic nitrogen fraction in the plants and the proportions of nitrogen bearing salts in the culture solutions, although in the

young plants of series A four of the five highest yields of this fraction were produced by plants grown in solutions containing relatively high proportions of ammonium nitrogen and low proportions of nitrate nitrogen.

Although the yields of insoluble organic nitrogen in these plants fluctuate less between cultures of a given age within a series than do any of the other nitrogen fractions here considered, they vary more than do the yields of any other fraction between plants of corresponding cultures at different ages. The nitrogen in this fraction of oat seedlings when 20 days old represented an average of 3.65 per cent of their total dry weight. When the plants were 40 days old, in the early heading stage, this form of nitrogen had decreased to an average of 3.07 per cent. Then, as the plants passed the heading stage and approached maturity, there came a rapid decline in the insoluble organic nitrogen, so that on the date of harvest of series C it had decreased to an average of 1.9 per cent. This decline may be accounted for by the fact that as the plants approach maturity an increasing number of cells lose their protoplasmic content and the insoluble organic nitrogen decreases correspondingly for a given dry weight of tissue.

Total organic nitrogen. The close relationship which exists between the ammonium nitrogen of the solutions and the total organic nitrogen of the plants is evident from the data of table 6. High yields of this fraction correspond with high proportions of nitrogen as ammonium in the culture solutions and, in general, low yields correspond to low proportions of ammonium in these solutions.

These data of table 6 indicate that nitrogen as ammonia is more closely associated with the synthesis of proteinaceous compounds in the plant than is nitrate nitrogen, although, as has been shown previously, the inorganic nitrogen of the plant is composed largely of nitrate nitrogen. To emphasize further these relationships, the values of the total organic and the total inorganic nitrogen fraction of the plants of series A, B, and C are diagrammatically represented in figure 4 together with the osmotic proportions of the nitrogen bearing salts in the culture solutions. At the left are given the percentage values of total inorganic nitrogen, and on the right those of the total organic fraction. This diagram is constructed in precisely the same manner as that of figure 3.

It will be observed from figure 4 that both the total organic and the total inorganic nitrogen fractions are higher in the younger than in the older plants. A comparison of the diagrams representing the inorganic nitrogen values in series A and B with the relative proportions of nitrogen as nitrate in the culture solutions, shows that almost without exception high and low values of total inorganic nitrogen in the plants correspond, respectively, with high and low relative proportions of nitrate nitrogen in the culture solutions. The opposite relation is shown between total inorganic nitrogen in the plants and nitrogen as ammonium in the culture solutions. It is clear, then, that the nitrate nitrogen in the culture solutions, and not the nitrogen as ammonium, deter-

mines to a very large extent the total inorganic nitrogen of the plants. This follows from the fact that at any time nitrogen as ammonium comprises a very small proportion of the total inorganic nitrogen of the plants. On the other hand, by comparing the total organic nitrogen in the plants of series A and B with the two forms of nitrogen in the solutions, it will be observed that with very few exceptions, high and low total organic nitrogen in the plants corre-

TABLE 6

Total organic nitrogen in plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBERS	OSMOTIC PROPORTIONS				TOTAL ORGANIC NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C
						per cent	*per cent*	*per cent*
1	12	3	3	1	3	4.20	3.20	2.71
	14	3	3	3	1	4.48	3.22	2.64
2	2	5	1	1	3	4.53	3.24	2.92
	5	5	1	3	1	4.63	3.81	3.34
3	18	1	5	1	3	4.12	5.55	2.41
	19	1	5	3	1	Lost	3.63	2.61
4	3	3	1	1	5	4.27	3.13	2.96
	8	3	1	5	1	5.06	4.23	3.32
5	13	1	3	1	5	4.65	3.23	2.46
	16	1	3	5	1	5.00	3.58	4.29
6	4	1	1	1	7	4.70	3.56	3.03
	10	1	1	7	1	5.25	4.22	Killed
7	7	1	1	3	5	4.91	4.06	3.03
	9	1	1	5	3	4.93	3.86	3.85
8	20	1	7	1	1	4.43	3.16	2.22
	1	7	1	1	1	4.15	3.32	2.69
9	17	3	5	1	1	4.63	3.32	2.36
	11	5	3	1	1	4.19	3.66	2.21
10	15	1	3	3	3	4.81	3.86	3.22
	6	3	1	3	3	4 72	3.58	3.24

spond, respectively, with high and low relative proportions of nitrogen as ammonium in the culture solutions, whereas nitrate nitrogen in the solutions appears to have less influence upon the total organic nitrogen of the plants.

In general, the relations here pointed out for the plants of series A and B hold also for those of series C, but in the older plants these relations are not

quite so exact as they are in younger plants. This is to be expected, since, as already shown, the protoplasmic content of the older plants is low, as is their nitrate nitrogen content, whereas nitrogen as ammonia accumulates in their tissues—sometimes to the extent of actual toxicity—and thus it comprises a greater percentage of the inorganic nitrogen of the plants of series C than of the younger plants in series A and B.

TABLE 7

Total nitrogen in plants of series A, B, and C

PAIRS OF CULTURES	CULTURE SOLUTION NUMBER	OSMOTIC PROPORTIONS				TOTAL NITROGEN		
		$MgSO_4$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Series A	Series B	Series C
						per cent	*per cent*	*per cent*
1	12	3	3	1	3	5.71	4.58	3.70
	14	3	3	3	1	6.29	4.27	4.09
2	2	5	1	1	3	6.47	4.78	4.19
	5	5	1	3	1	6.43	4.91	4.47
3	18	1	5	1	3	6.12	5.39	3.81
	19	1	5	3	1	6.61	5.06	4.09
4	3	3	1	1	5	6.65	4.91	4.27
	8	3	1	5	1	6.80	5.45	4.91
5	13	1	3	1	5	6.63	4.62	3.87
	16	1	3	5	1	6.88	4.81	5.52
6	4	1	1	1	7	6.93	5.54	4.46
	10	1	1	7	1	7.00	5.45	Killed
7	7	1	1	3	5	7.08	5.80	4.84
	9	1	1	5	3	6.87	5.34	5.87
8	20	1	7	1	1	6.18	4.45	3.32
	1	7	1	1	1	5.79	4.46	3.84
9	17	3	5	1	1	6.47	4.85	3.39
	11	5	3	1	1	6.99	4.79	3.39
10	15	1	3	3	3	6.94	5.34	4.49
	6	3	1	3	3	6.97	4.96	4.77

Total nitrogen. The total nitrogen includes both the organic and the inorganic nitrogen fractions of the plants. The data relating to total nitrogen are presented in table 7. As previously shown, when the inorganic nitrogen of the plants has relatively high values the organic nitrogen is relatively low, and thus the total nitrogen of the plants within a given series fluctuates less than does any one of the fractions included in the total nitrogen. Since the

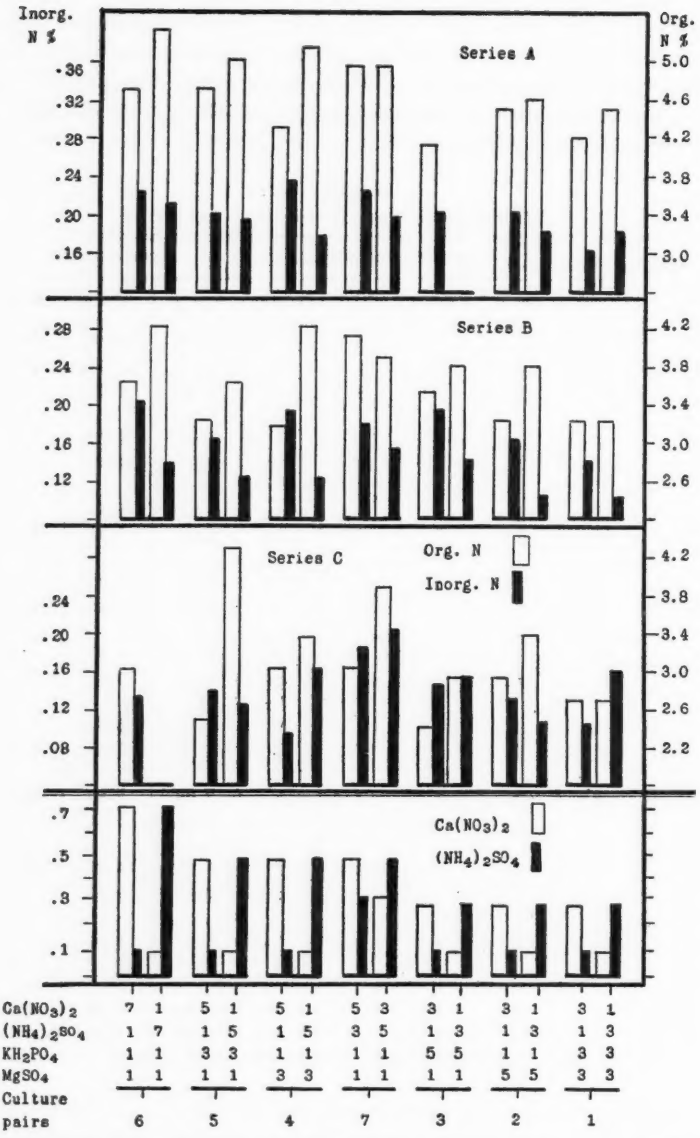

FIG. 4. DIAGRAM SHOWING THE RELATION BETWEEN THE OSMOTIC PROPORTIONS OF THE NITROGEN BEARING SALTS OF THE CULTURE SOLUTIONS AND THE TOTAL INORGANIC AND THE TOTAL ORGANIC NITROGEN IN THE PLANTS

larger part of the total nitrogen of the plants is in the organic form, and since organic nitrogen is closely correlated with nitrogen as ammonium in the culture solutions, it follows that the total nitrogen of the plants is influenced considerably more by the ammonium nitrogen than by the nitrate nitrogen of the solutions, although the latter exerts considerable influence in determining the total nitrogen of the plants, since the inorganic nitrogen fraction of the plants is largely determined by the nitrate nitrogen in the culture solution in which the plants were grown.

From a physiological point of view, the total nitrogen of the plant carries little significance, since it gives no clue to either the type or the quantity of nitrogen in any particular nitrogen fraction of the plant, and it is quite clear that it can not serve as an adequate criterion of any organic constituent of the plant when, as frequently found, more than one-third of the total nitrogen present in the plants may be inorganic in nature, largely in the form of nitrate nitrogen.

From the foregoing considerations, it is apparent that ammonium nitrogen is more closely associated with the synthesis of the organic nitrogenous compounds of the plant than is nitrate nitrogen. In the early stages of growth, when protein synthesis per gram of tissue is most rapid, the ammonia nitrogen is undoubtedly readily converted into the organic forms of nitrogen and very little of it accumulates in the tissue of the younger plants. Thus the plants in the solutions with high relative proportions of nitrogen as ammonium and low relative proportions of nitrate nitrogen made just as good growth as, and had a higher protein content and a lower content of inorganic nitrogen during the early phases of their development than, did the plants in solutions with low relative proportions of nitrogen as ammonium and high relative proportions of nitrogen as nitrate. In the later stages of growth when the plants are approaching maturity, the synthesis of nitrogenous compounds per gram of tissue is greatly reduced as compared with this process in younger plants. Thus the conversion of ammonia nitrogen to the organic forms is greatly retarded. In the older plants, grown in solutions with high relative proportions of nitrogen as ammonium and low relative proportions of nitrogen as nitrates, absorption of ammonium nitrogen apparently outstripped assimilation, with the result that ammonia nitrogen accumulates in the plant tissues in sufficient concentrations to retard growth materially, and sometimes to produce severe pathological conditions, whereas the same solutions often produce much better plants during the early stages of development than do solutions with high relative proportions of nitrogen as nitrate and low relative proportions of ammonium nitrogen.

SUMMARY

From the investigation reported in the foregoing pages, on the quantitative relationships between the inorganic nitrogen constituents of culture solutions of known composition and the various nitrogen fractions of oat plants produced by these solutions, the following relations may be briefly summarized:

In the younger stages of development, the oat plants grew equally well in the high ammonia and in the high nitrate solutions, but in the later stages of their development the growth of the plants in solutions high in ammonium was much less than that of plants in the high nitrate solutions.

Correlated with low dry weight yields of the older plants in the high ammonium solutions was a relatively high yield of nitrogen as ammonia in the plants, and this was the only nitrogen fraction considered which showed higher yields in the older than in the younger plants.

The nitrogen as ammonia in the plants was high or low, respectively, with high or low proportions of nitrogen as ammonium in the culture solutions in which the plants were grown.

The nitrate nitrogen in the plant was high or low, respectively, with a high or low amount of nitrate nitrogen in the culture solutions in which the plants were grown.

The inorganic nitrogen of these plants was largely in the nitrate form. It often represented more than 2 per cent of the dry weight of the plants and sometimes comprised more than one-third of the total nitrogen.

Plants high in soluble organic nitrogen had high ammonia content and were produced by solutions with high relative proportions of nitrogen as ammonium and low proportions of nitrate nitrogen.

For plants of a given age, the insoluble organic nitrogen fraction was more constant and less influenced by the chemical composition of the culture solutions in which the plants were grown than were any of the other nitrogen fractions; but since protein synthesis per gram of tissue was greatly reduced as the plants approached maturity, the insoluble organic nitrogen was more influenced by the age of the plant than was any other fraction.

Solutions with high relative proportions of nitrogen as ammonium produced plants which were high in total organic nitrogen and low in inorganic nitrogen, whereas solutions with high relative proportions of nitrate nitrogen produced plants high in inorganic and low in organic forms of nitrogen.

The total nitrogen of the plants varied with the total nitrogen of the solutions in which the plants were grown, but not in direct proportion to the amounts appearing in the culture solutions.

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STUDIES ON NITROGEN ABSORPTION FROM CULTURE SOLUTIONS: I. OATS[1]

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The investigations described in the following pages deal with a study of the absorption of nitrogen in the forms NH_4 and NO_3 by roots of plants grown in solution cultures.

The status of this problem of the utilization of ammonium and nitrate nitrogen by agricultural plants is still in a very unsatisfactory condition. Experimental evidence favors the idea that not only are ammonium salts as well as nitrates good sources of nitrogen for higher plants, but also that both forms are required under certain conditions by some plants and that plants differ in their ability to utilize ammonium as a source of nitrogen. Some plants appear to require ammonium during one stage of development and nitrate during another.

A review of the literature on this subject shows that the substitution of ammonium nitrogen, either partially or wholly, for nitrate nitrogen is a procedure which is not of recent origin. Hutchinson and Miller (7, 8) summarize the more definite contributions to the literature on this subject, as do Jones and Shive (10).

More recently Prince, Jones, and Shive (13) have shown that when ammonium sulfate is superimposed upon the Shive three-salt solution, or when this salt is substituted for the potassium nitrate of the Tottingham solution in equivalent osmotic concentrations, thus enabling the plants to obtain their nitrogen in the two forms, NH_4 and NO_3, the H-ion concentrations of the solutions in which the roots of the absorbing plants are immersed increase during the early stages of the growth of the plants, but the rate of increase is not nearly so marked as it is when NH_4 alone furnishes the source of nitrogen. However, when the plants reach a certain stage in their development, the direction of the reaction change is reversed, thus indicating a reversal in the NH_4 and NO_3 absorption rates. This occurs when the plants are 4 or 5 weeks old.

Jones and Skinner (9) in a recent investigation found that the relative absorption rate of ammonium nitrogen per unit area of absorbing surface decreases with the age of the plant, although it increases with respect to nitrate nitrogen absorption. They also observed that the rate of absorption of ammonium nitrogen does not materially increase with an increase in concentration of the ammonium salt in the solution, whereas, on the other hand, the rate of nitrate nitrogen absorption does increase markedly as the nitrate content of the medium increases.

The following investigations were undertaken for the purpose of studying further the relative rates of the absorption of nitrogen in these two forms,

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NH_4 and NO_3, by the roots of plants throughout the entire life cycle, when both forms are present in the culture solution and available to the plants. The studies were carried out with two species: oats (*Avena sativa*) and buckwheat (*Fagopyrum esculentum*). The results obtained with oats alone will be presented here; those obtained with buckwheat will appear in a later publication.

EXPERIMENTAL METHODS

Method of growing plants

A pure line of oat seeds of the Gopher variety were germinated on a net as described by Shive (14). Seedlings, carefully selected for uniformity of size and vigor, were transferred when about 5 cc. tall to a culture solution, after having been mounted in a double piece paraffined cork stopper devised by Tottingham (16). The stoppers were of the proper size to fit 2-quart fruit jars of colorless glass, which were used for culture vessels. Three seedlings were included in each culture. The cultures were grown in the green-house with full exposure to sunlight, the temperature ranging between 18°C. and 23°C.

The culture media for all the work were the modified Tottingham solution $T_1R_3C_5$ (10) containing $(NH_4)_2SO_4$, $Ca(NO_3)_2$, KH_2PO_4, and $MgSO_4$ in the following volume molecular proportions: 0.0042, 0.0075, 0.0021, and 0.0024, respectively; and solution $T_1R_3C_3$ with the volume molecular proportions of 0.0042, 0.0043, 0.0021, and 0.0071, respectively. Baker's analyzed salts were used in the preparation of all the solutions. The solutions were renewed continuously by the addition of 1 liter of fresh solution every 24 hours according to the "drip and drain" method at constant rates described by Shive and Stahl (15). This system of solution renewal also aerates the solution. Iron was supplied daily to all the cultures in the form of freshly prepared aqueous solution of ferrous sulfate, according to the need of the plant determined by its appearance. Boron was added in the form of boric acid in concentration of 1 p.p.m. In all cases, the solution used and the methods employed in the cultural procedure permitted an excellent development of the plants. The cultures were prepared and conducted as described in the foregoing through a time period of 12 days before the first absorption test was made.

Method of transferring cultures for the absorption tests

When the plants were 18 days old from the time of germination and 12 days in the culture jars, 24 cultures were selected in which the plants were about equal with respect to size and vigor in so far as this could be judged from careful observation. Two groups of 12 cultures each were selected. The three plants in each culture, together with the paraffined cork stoppers in which they were mounted, were taken from the 2-quart culture jars and transferred to pint jars containing exactly 500 cc. of the same solution as that in which

the plants were grown. By the continuous flow method of Shive and Stahl (15) 1 liter of new solution was then passed through each culture jar containing the measured 500 cc. of solution, in which the roots of the plant were immersed, during the absorption interval. The continuous flow method was necessary for reasons which will be made clear in a following section. Before the plants were transferred to the measured solutions, the roots were thoroughly rinsed with distilled water. This transfer of all the plants in both groups of a series was made at the time when the experiment began, always at 8:00 a.m. One culture was then removed from each group at the end of 4 hours, another at the end of 8 hours, and so on at 4-hour intervals up to 48 hours.

At the end of each absorption interval the plants were harvested and their green and dry weights obtained in the usual way. A new series of cultures was then started as before and the plants grown as before until they were 12 days older than those of the series which immediately preceded. This procedure was continued until the last series to be investigated was at or near the stage of maturity, when the absorption tests were made.

Methods of analyses

Immediately after the removal of the cultures from the series at the end of the various experimental periods, the solutions in which the plant roots had been immersed for definite intervals, together with two solution checks and a distilled water check, were analyzed for ammonium nitrogen, nitrate nitrogen, and total nitrogen. A 100-cc. aliquot was used for each analysis. The methods used were those which, after many preliminary tests, were found best adapted to determine small amounts of inorganic nitrogen, and which gave very small analytical error. Several methods were tried, but the experimental error in some of these was relatively high and could not compare with the following methods for accuracy:

Ammonium nitrogen determination. The Official Kjeldahl-Gunning-Arnold Method. (Association of Official Agricultural Chemists) (1) was employed with modifications in apparatus and quantities of distillate, acid and water used, as recommended by Paul and Berry (12).

Nitrate nitrogen determination. The Devarda Method [Association of Official Agricultural Chemists (2)], with modifications by Paul and Berry (12) as suggested in the foregoing.

The ammonium nitrogen was determined on a 100-cc. aliquot, cooled, and the reductor (Devarda's Alloy Metal containing Cu, 50 per cent; Zn, 5 per cent; and Al, 45 per cent, was added and the nitrate nitrogen determined following the official method as stated in the foregoing.

Total nitrogen determination. The total nitrogen was determined by the official method of the Association of Official Agricultural Chemists (3) and checked by adding together the ammonium and nitrate nitrogen values. All analytical values were checked very carefully with the theoretical values,

whenever this was possible, and if these did not correspond very closely the values were discarded. If the analytical values for the duplicate cultures did not check very closely with each other, these values were discarded. In general the values for duplicate cultures did check very closely, showing that differences in the rates of absorption between the cultures was not a disturbing factor in these experiments.

The acid and alkali used in titration were standardized against recrystallized Merck's Blue Label calcium carbonate. Twentieth normal acid and alkali were used in all titrations and the normality factors determined frequently to avoid possible error from this source. All titrations were carried out with a Schellbach burette and to complete disappearance of any red color. With twentieth normal alkali and acid, methyl red was found to be much more sensitive than cochineal or methyl orange as indicators.

Every determination was run in duplicate and repeated if checks were not obtained. The distilled water checks showed no nitrogen present in either the ammonium or the nitrate forms.

The periods at the different stages of development of the plants during which these experiments were carried out, will here be designated as *experimental periods*, and the time periods of immersion of the roots in the test solutions will be designated as *test* or *absorption intervals*.

Since duplicate cultures were employed during each absorption interval and since all analyses were carried out in duplicate, all the values represented in the tables are averages of at least four determinations involving two cultures and six plants. Since 24 cultures constituted a series for every experimental phase, all the final average values presented in the tables and those plotted and represented in graphs are averages of 48 determinations each involving 24 cultures and 72 plants.

All results were calculated (*a*) on the basis of actual nitrogen absorbed in the form in question, in milligrams per culture of three plants per hour, which will here be designated *quantity absorption*, and (*b*) on the basis of milligrams per gram of dry plant material per hour, which will be designated *rate absorption*. The data obtained by these two methods of calculation will be presented and considered separately.

EXPERIMENTAL RESULTS

Preliminary experiments

During some preliminary experiments, plants were transferred from the 2-quart culture jars in which they were grown with a continuously renewed solution, to the pint jars containing 500 cc. of solution, where they remained for the time of the absorption interval without continuous flow of solution through the culture jar. At the end of the test interval the plants were returned to the original 2-quart culture jars after the roots had been rinsed free from adhering solution with distilled water from a wash bottle, care being

taken to retain all the rinse water with the solution to be analyzed. Here they were grown for another 12 days in a continuously renewed solution, when a similar transfer and test was made. This procedure was continued at 12-day intervals throughout the course of these preliminary experiments, extending over a growth period of 78 days when the plants had just passed the blossoming stage. The modified Tottingham solution $T_1R_3C_5$ (10) was used throughout. Because of the water loss due to transpiration, distilled water was added to the culture jars at frequent intervals to maintain as nearly as possible the original volume of the test solution.

All results were here calculated only on the basis of nitrogen absorbed (removed from a fixed quantity of solution) in the form in question, in milligrams per culture per hour (quantity absorption). Since the plants were not harvested at the end of each test interval, dry weights could not be obtained upon which to calculate absorption rates on the basis of nitrogen absorbed per gram of dry plant material per hour.

Some of the data dealing with these preliminary experiments are here presented briefly for the sole purpose of bringing out and emphasizing several important points, the principal one of which deals with the influence of continuous renewal of test solutions, or the lack of it, upon absorption rates during the test intervals. These data are presented in table 1. As may be observed, each horizontal section of the table presents the data obtained during an experimental period including 12 absorption intervals ranging in time from 4 hours as the minimum to 48 hours as the maximum time during which the roots of the plants were exposed to the test solution. Both absorption tests and analytical determinations were carried out in duplicate; hence each value in the table except those in the last line of each horizontal section represents the average of four determinations. Each of the values in the last line of each horizontal section, marked *average*, represents the final average of 48 determinations involving 24 cultures and 72 plants.

The final average values for all the experimental growth stages as given in table 1, were plotted to form the graphs of figure 1. It will be observed that the graph representing the average hourly quantity intake of nitrogen as NH_4 shows a gradual decrease in values for the experimental stages until the plants had reached the age of 66 days, at which point the graph takes an abrupt rise. This indicates that up to this point the average hourly intake of nitrogen as NH_4 gradually decreases with the age of the plant. The abrupt break in the curve following the 66-day experimental stage immediately followed abundant tillering of the plants and the formation of much new and vigorous young tissue. The abrupt rise in this graph is undoubtedly the result of the formation of this young plant tissue with consequent increased nitrogen intake as NH_4 per culture per hour, since it is evident that under the conditions of these tests young growth absorbs nitrogen as NH_4 at a much higher rate than does the older growth. Hence, tillering, which represents young growth, abruptly reverses the direction of the graph of NH_4 absorption.

TABLE 1

Quantity absorption of nitrogen as NH_4 and NO_3 and total nitrogen per culture by oat plants during absorption intervals ranging in time from 4 hours to 48 hours, at different stages of development in the life cycle

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		NH_4, per culture		NO_3, per culture		Total N, per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
18	4	2.900	0.725	−0.444	−0.111	2.456	0.614
	8	4.188	0.524	−0.222	−0.028	3.966	0.496
	12	4.461	0.372	−0.111	−0.009	4.350	0.363
	16	4.961	0.210	−0.111	−0.007	4.850	0.203
	20	5.353	0.268	0.233	0.012	5.586	0.280
	24	5.688	0.237	0.667	0.028	6.357	0.265
	28	5.911	0.211	2.450	0.088	8.361	0.299
	32	6.002	0.188	2.004	0.063	8.006	0.251
	36	5.911	0.164	2.227	0.062	8.138	0.226
	40	5.799	0.145	3.006	0.075	8.805	0.220
	44	5.688	0.129	3.340	0.076	9.028	0.205
	48	5.799	0.121	4.230	0.088	10.029	0.209
Average			0.275		0.028		0.303
30	4	2.656	0.664	−0.273	−0.068	2.383	0.596
	8	3.149	0.394	−0.796	−0.099	2.353	0.295
	12	3.237	0.270	−0.441	−0.037	2.796	0.233
	16	3.415	0.213	0.276	0.017	3.691	0.230
	20	3.680	0.184	0.826	0.041	4.506	0.225
	24	4.034	0.168	1.419	0.059	5.453	0.227
	28	4.389	0.157	1.950	0.070	6.339	0.227
	32	4.831	0.151	1.773	0.055	6.604	0.206
	36	4.743	0.132	1.535	0.043	6.278	0.175
	40	4.831	0.121	2.127	0.053	6.958	0.174
	44	5.160	0.117	3.103	0.071	8.263	0.188
	48	4.983	0.104	3.634	0.076	8.617	0.180
Average			0.223		0.023		0.246
42	4	2.932	0.733	0.178	0.045	3.110	0.778
	8	3.502	0.438	−0.178	0.022	3.324	0.416
	12	4.114	0.343	−0.711	0.059	3.403	0.284
	16	4.114	0.257	−0.888	0.056	3.226	0.201
	20	5.446	0.272	−0.617	0.031	4.829	0.241
	24	5.859	0.244	0.000	0.000	5.859	0.244
	28	6.652	0.238	2.689	0.096	9.341	0.334
	32	5.509	0.172	3.376	0.106	8.885	0.278
	36	5.261	0.142	7.122	0.198	12.383	0.344
	40	5.261	0.132	11.103	0.278	16.364	0.410
	44	4.639	0.105	12.436	0.283	17.075	0.388
	48	4.639	0.097	12.791	0.267	17.430	0.364
Average			0.265		0.092		0.357

TABLE 1—*Concluded*

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		NH_4, per culture		NO_3, per culture		Total N, per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
54	4	1.518	0.380	−0.076	−0.019	1.442	0.361
	8	2.592	0.324	−0.132	−0.017	2.460	0.307
	12	3.037	0.253	2.978	0.248	6.015	0.501
	16	3.704	0.232	5.385	0.337	9.089	0.569
	20	3.333	0.107	5.755	0.288	9.088	0.395
	24	2.963	0.123	5.940	0.248	8.903	0.371
	28	2.592	0.093	6.607	0.236	9.199	0.329
	32	2.407	0.075	7.792	0.244	10.199	0.319
	36	2.592	0.072	10.015	0.278	12.607	0.350
	40	2.222	0.056	10.383	0.259	12.605	0.315
	44	2.073	0.047	11.309	0.257	13.382	0.304
	48	2.222	0.046	11.766	0.245	13.988	0.291
Average			0.151		0.217		0.368
66	4	0.504	0.126	5.023	1.256	5.527	1.382
	8	0.511	0.064	8.513	1.064	9.024	1.128
	12	0.507	0.042	10.652	0.888	11.159	0.930
	16	0.536	0.034	10.968	0.686	11.504	0.720
	20	0.511	0.026	11.099	0.555	11.610	0.581
	24	0.741	0.031	11.344	0.473	12.085	0.504
	28	1.926	0.069	10.009	0.357	11.935	0.426
	32	2.362	0.074	10.297	0.322	12.659	0.396
	36	4.228	0.117	11.629	0.323	15.857	0.440
	40	4.611	0.115	12.258	0.306	16.869	0.421
	44	4.228	0.096	12.693	0.288	16.921	0.384
	48	3.768	0.079	13.422	0.280	17.190	0.359
Average			0.073		0.567		0.639
78	4	2.932	0.733	2.449	0.612	5.381	1.345
	8	3.501	0.438	3.330	0.416	6.831	0.854
	12	3.344	0.279	4.342	0.362	7.686	0.641
	16	4.402	0.275	5.097	0.319	90.499	0.594
	20	5.381	0.268	6.365	0.318	11.746	0.586
	24	5.859	0.244	8.078	0.337	13.937	0.581
	28	5.859	0.209	11.750	0.420	17.609	0.629
	32	5.509	0.172	13.953	0.436	19.462	0.608
	36	5.461	0.152	15.177	0.422	20.638	0.574
	40	5.461	0.137	15.666	0.392	21.127	0.529
	44	4.640	0.105	16.646	0.378	21.286	0.483
	48	4.639	0.097	17.625	0.367	22.264	0.464
Average			0.259		0.398		0.657

On the other hand, the graph representing the absorption of nitrogen as NO_3 begins with very low values and gradually rises with increase in the age of the plants, reaching a maximum at the 66-day experimental stage, when the graph takes an abrupt downward direction which corresponds to the pronounced upward direction taken by the graph representing nitrogen absorption as NH_4 and coincides with the development of numerous tillers and abundant young growth by the plants. Thus during the early stages of growth a low quantity intake of nitrogen as NO_3 is indicated for the oat plant and a relatively high intake per culture per hour during the later stages of growth.

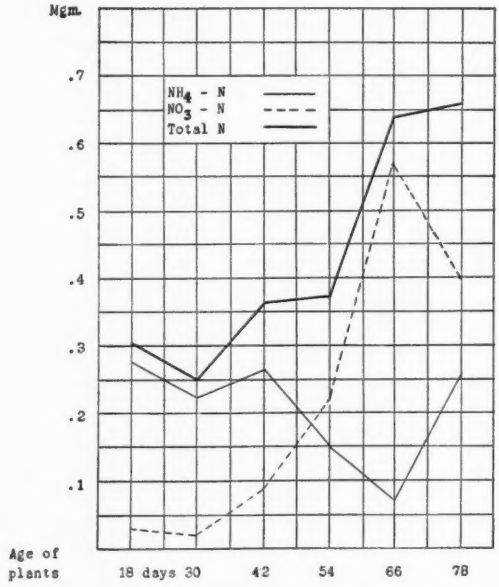

FIG. 1. GRAPHS REPRESENTING QUANTITY OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN ABSORBED, IN MILLIGRAMS PER CULTURE PER HOUR, AT DIFFERENT PERIODS IN THE LIFE CYCLE OF THE OAT PLANT

The graph representing total nitrogen intake per culture per hour takes a general upward slope from the point representing the first experimental growth stage to the point representing the last stage, indicating that the total nitrogen intake gradually increases with the development of the plants to the 78-day stage, when the plants had just passed through the flowering period. An accident necessitated the discontinuance of this series of cultures at the 78-day stage. However, as will be shown later, total nitrogen intake per culture per hour reaches its maximum for this plant at about this stage of development.

Irregularities in the graphs during the early growth phases may be traced to external conditions, particularly with respect to light intensity and temperature during the test periods, although no attempt was here made to measure the influence of these factors upon nitrogen absorption.

The important point to be emphasized here is the apparent high ability of vigorous young tissue, occurring either as the seedling phase of the life cycle or produced at a later stage of development, to absorb nitrogen as NH_4 almost to the exclusion of nitrogen as NO_3, when the two forms occur simultaneously in the culture medium. Equally interesting and important is the fact that the plants appear to lose this ability rapidly and apparently acquire increasing power to absorb nitrogen as NO_3 with increasing age of the tissues under the experimental conditions here defined. This latter point will be brought out more clearly and emphatically in a following section with a consideration of the actual absorption rates calculated on the basis of unit weight of dry plant tissue.

Influence of duration of absorption interval upon absorption rates

Absorption from a fixed quantity of solution without continuous solution renewal. The data of table 1 reveal the fact that during any one experimental period of 48 hours in the life cycle the quantity of nitrogen absorbed in milligrams per culture per hour decreases very markedly as the time of the absorption interval increases, except when the absorption values are very low. This occurs when absorption takes place from a fixed quantity of solution, limited in volume, without continuous renewal of solution. To illustrate these relations conveniently and clearly, the quantity absorption values per culture per hour as given in table 1, for the experimental periods at 18 days and at 66 days have been plotted to form the two sets of graphs given in figure 2. The data for only these two experimental periods are here presented in graphic form since they are representative of the data obtained for all the experimental periods during the life cycle of the plants, and the data for the remaining periods may very well be omitted from the discussion without detracting from the general significance of the results indicated by the graphs of figure 2.

As previously described, the cultures, each consisting of three plants, were placed in half-liter jars each containing 500 cc. of solution where they remained during the respective absorption intervals without solution renewal. At the end of the intervals the plants were removed, the solutions analyzed, and the absorption data obtained.

From the graphs corresponding to the experimental period at 18 days it will be observed that the quantities of nitrogen absorbed as NH_4 per culture per hour gradually decrease as the length of the absorption intervals increases. On the other hand, the corresponding NO_3 absorption values show only slight variation as the length of the absorption interval increases. The NO_3 values throughout this experimental period were very low, whereas the NH_4 values were relatively high, as indicated by the graphs.

The graphs representing the corresponding data for the experimental period when the plants were 66 days old appear in a position which is just the reverse of that of the preceding graphs for the period at 18 days. Here the graphs of

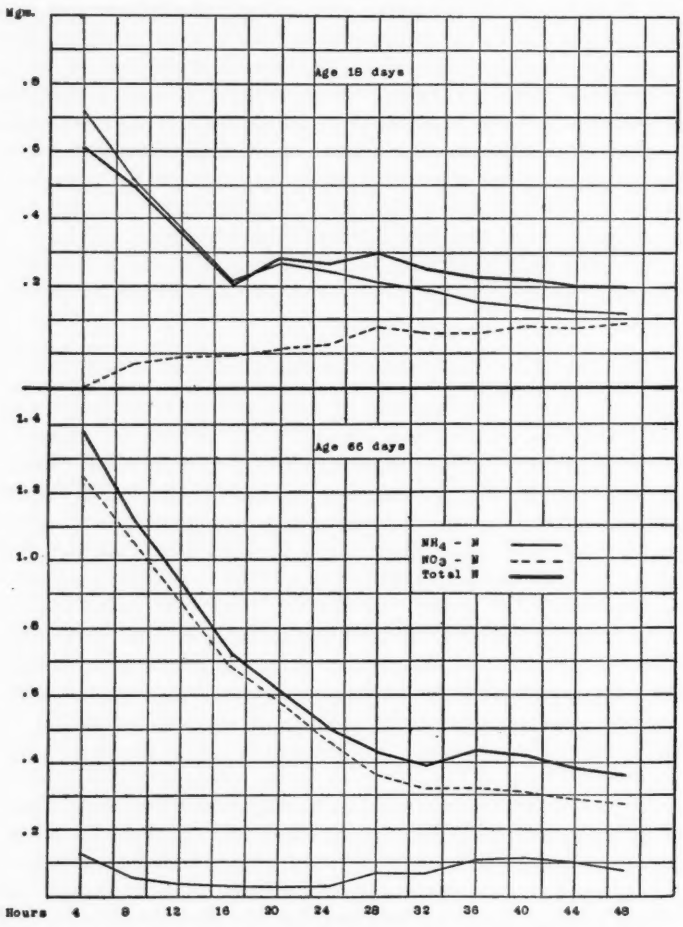

FIG. 2. GRAPHS SHOWING THE INFLUENCE OF DURATION OF ABSORPTION INTERVALS ON NITROGEN ABSORPTION RATES WITHOUT CONTINUOUS RENEWAL OF CULTURE SOLUTIONS

the absorption quantities of NO_3 in milligrams per culture per hour show values which are much higher than those of the corresponding NH_4 values throughout the entire experimental period of 48 hours. The average hourly NO_3 values now gradually decrease as the time of the absorption intervals in-

creases. During this experimental period the NH_4 values are all noticeably low and show only slight differences.

From a consideration of the graphs of figure 2, it appears that whenever the two forms of nitrogen here employed occur simultaneously in the culture solution and one form is absorbed by the plant roots from a fixed quantity of solution, without continuous renewal, in relatively low quantities and the other in relatively high quantities, the average hourly absorption of the latter decreases markedly with increase in the length of the time interval of absorption while that of the former is either not at all or only slightly influenced by variations in the length of the absorption interval. This is perhaps what might be expected on the assumption that change in concentration in the external medium through absorption of the particular constituent in question correspondingly changes the hourly absorption of this constituent during any given experimental period. But the matter appears to be not so simple as this. There can be no disputing the fact that quantity absorption per culture per hour is very materially influenced also from interval to interval by external variable factors such as light intensity in particular, temperature, and humidity, and this has been the main reason for adopting, in these investigations, a relatively large number of test intervals with a very wide range in time values (4 to 48 hours) during each experimental period throughout the life cycle of the plants. No attempt has here been made, of course, to evaluate these factors in their relation to nitrogen absorption.

As has already been pointed out, quantity absorption of nitrogen per calture per hour in the two forms here used, form identical solutions, changed markedly with the age of the plant, as is indicated by the complete reversal of the position of the graphs representing NH_4 absorption and NO_3 absorption when the plants were 18 days old and when they were 66 days old; but this is not a question of concentration. That the change in concentration of the nitrogen or other elements in the external medium due to absorption by the plant roots is the main factor which brings about this variation in the average hourly absorption with variable absorption intervals during an experimental period of 48 hours is clearly brought out by a similar consideration of corresponding data of absorption from fixed quantities of solution but continuously renewed during the absorption intervals.

Absorption from a fixed quantity of solution with continuous solution renewal. The data represented by the graphs of figure 3, correspond in every respect with those represented by the graphs of figure 2, except that the former were obtained from cultures in which the plant roots of each were immersed in a fixed quantity of solution which was continuously renewed during the absorption intervals, while the latter were obtained from cultures in which the roots of the plants were exposed to the same fixed quantity of solution but without continuous flow during the test intervals.

The absorption tests were here made by immersing the plant roots of each culture in 500 cc. of solution as before and then passing through the culture

jar, by the method of continuous flow at a constant rate described by Shive and Stahl (15), 1 liter of new solution during the absorption interval. The solution drained from the culture jar during the test interval was caught and

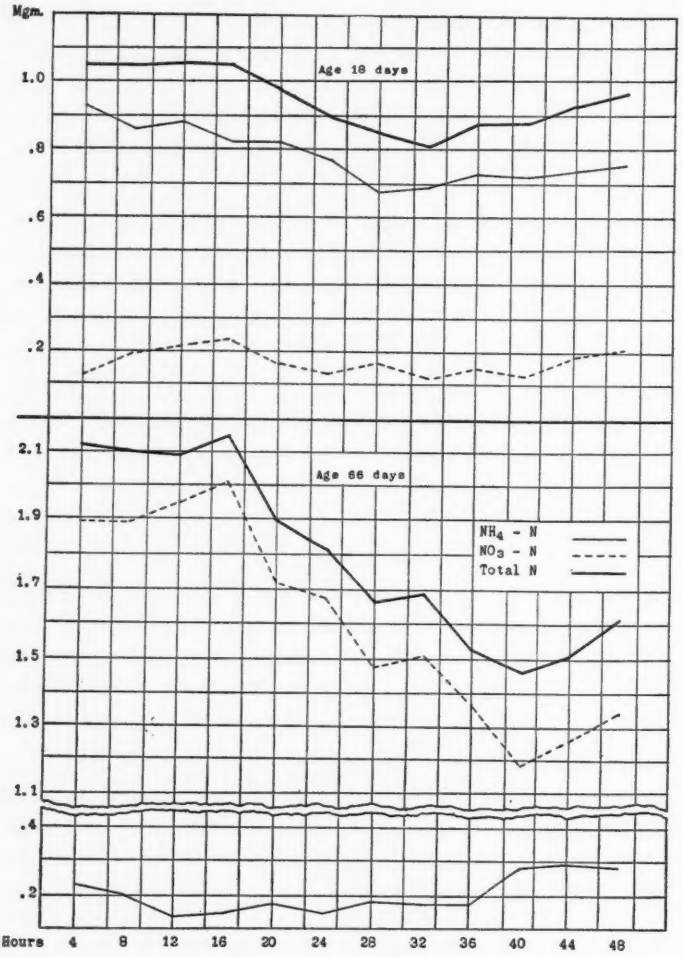

FIG. 3. GRAPHS SHOWING THE INFLUENCE OF DURATION OF ABSORPTION INTERVALS ON NITROGEN ABSORPTION RATES WITH CONTINUOUS RENEWAL OF CULTURE SOLUTIONS

added to the 500 cc. remaining in the culture jar at the end of the test. Analyses were then made on the whole 1,500 cc. of test solution. This method was used in all of the work here reported except that of the preliminary experiments already described.

The data, for the experimental period of 48 hours at 18 days and at 66 days, represented by the graphs of figure 3, were taken from table 2, and correspond to the data represented by the graphs of figure 2 taken from table 1.

From the graphs of figure 3, representing the data for the 18-day experimental period, it will be observed that with continuous flow there is no significant variation in the nitrogen absorption in milligrams per culture per hour with increase in length of time of the test intervals. This is indicated by the approximately horizontal position of the graphs.

The graphs representing the data for the 66-day period show a slight decrease in the absorption of total and NO_3 nitrogen in milligrams per culture per hour as the time duration of the test intervals increases, as is indicated by the downward pitch of the graphs following the 16-hour absorption interval. Assuming, of course, that decrease in the absorption values indicated by the graphs is the result of change in concentration due to absorption, this shows that continuous flow of solution, even at the rate here employed, does not sufficiently overcome concentration change during the longer test intervals to counteract completely its retarding influence upon absorption rates. Here, of course, total and NO_3 absorption values are considerably higher than are total and NH_4 absorption values during the 18-day period.

Here again, as previously, the relative positions of the graphs indicate that total nitrogen absorption is dominated by NH_4 absorption during the early stages of development—the 18-day period—, whereas during the later growth phases—the 66-day period—, NO_3 absorption dominates total nitrogen absorption.

A comparison of the graphs of figure 2 with the corresponding graphs of figure 3 brings out very clearly the importance of some system of continuous flow solution renewal during investigations of the nature here described, and the data represented by these two sets of graphs are here presented to emphasize this importance. There can be little doubt that continuous flow is very important for the growth of experimental plants in general where culture solutions or sand cultures supplied with culture solutions are involved.

Consideration of nitrogen excretion. In table 1 some negative values are indicated for quantity absorption of NO_3-nitrogen during several experimental periods in the life cycle. These negative values are usually shown for the short time test intervals only, and appear to indicate excretion of nitrogen from the plant roots into the test solution. The regular occurrence of this phenomenon throughout the preliminary investigations without continuous solution renewal during the test intervals with both oats and buckwheat, when a fixed quantity of test solution (500 cc.) was employed with each culture, is fairly good evidence that these negative values, although small, are not purely accidental or due to experimental error, but probably have some significance.

It has been suggested (4) that under such conditions it is probable that some of the external root cells, as they die, and are sloughed off, give up their

solutes to the surrounding solution. It has also been suggested (5, 6) that the ions given off by the plant roots may be the result of leaching from the dead cells or exchange of ions in the cell wall or protoplasm. Whether these suggestions could explain the presence of the excess nitrogen found in the test solutions after contact with the plant roots, is questionable. At present no explanation of the phenomenon here observed can be offered.

It is important to point out, however, that in the absorption series to be described in the following sections, in which the method of continuous solution renewal at constant rates (15) was employed during the absorption intervals while the plant roots were in contact with the test solutions negative absorption (excretion) of nitrogen did not occur. Thus the importance of negative values in this connection largely disappears.

The absorption series

Introductory. The plants of these series were tested at seven different stages of development throughout the life cycle. This required seven different series of cultures, since the plants were harvested and their dry weights obtained at the end of each experimental period. Each series consisted of 12 cultures of three plants each, and each series was conducted in duplicate. The plants of the first series were tested when the plants were 18 days old. Those of the second series were tested at the age of 30 days, and so on at 12-day intervals, throughout the life cycle. The tests of the last series were made when the plants were 102 days old, having produced mature seed.

As in the preliminary experiments, each test extended over a period of 48 hours, the absorption intervals during each test ranging in time from 4 hours, which was the minimum time of exposure, to 48 hours, which was the maximum absorption interval as previously described. The modified Tottingham solution $T_1R_3C_3$ (10), which contained approximately equal proportions of nitrogen as NH_4 and as NO_3, was used as the growth medium of the plants in each of the seven series and also as the test solution during the seven experimental periods in the life cycle. Throughout the test intervals the plant roots of each culture were immersed in a fixed quantity (500 cc.) of solution which was continuously renewed by passing through the culture jar at a constant rate of flow 1,000 cc. of new solution during the respective test interval. The total quantity, 1,500 cc. of solution, to which the roots of a culture were exposed during the test interval was used in making the chemical analyses.

The results of the chemical analyses were calculated, first, on the basis of absolute quantities of nitrogen absorbed in the different forms per culture per hour. Since the plant as a whole is a variable quantity, increasing in size and weight with age, results obtained by this method indicate only the course of quantity nitrogen absorption during the life cycle. The results were calculated, second, on the basis of nitrogen absorbed in the different forms per unit quantity of dry plant tissue per hour. This method of calculation deals with actual absorption rates and indicates the activity of the

TABLE 2

Quantity absorption of nitrogen as NH_4, NO_3, and total nitrogen per culture by oat plants during absorption intervals ranging in time from 4 hours to 48 hours, at different stages of development throughout the life cycle

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		Ammonia per culture		Nitrate per culture		Total nitrogen per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	4	3.681	0.928	0.532	0.133	4.214	1.054
	8	6.837	0.855	1.596	0.199	8.433	1.054
	12	10.518	0.877			10.518	0.877
	16	13.148	0.821	3.723	0.233	16.871	1.054
	20	16.304	0.815	3.191	0.160	19.469	0.975
	24	18.407	0.767	3.191	0.133	21.599	0.900
18	28	18.933	0.676	4.786	0.171	23.720	0.848
	32	22.088	0.690	3.723	0.116	25.811	0.807
	36	26.295	0.730	5.319	0.148	31.614	0.878
	40	28.925	0.723	5.319	0.133	34.244	0.856
	44	32.660	0.741	8.510	0.193	41.117	0.934
	48	36.287	0.756	10.106	0.211	46.393	0.967
Average			0.780		0.152		0.934
	4	3.156	0.789	1.596	0.399	4.752	1.188
	8	7.889	0.986	3.096	0.387	10.985	1.373
	12	8.415	0.701	4.256	0.355	12.671	1.056
	16	7.889	0.493	3.629	0.227	11.518	0.720
	20	11.570	0.579	4.787	0.239	16.357	0.818
	24	15.251	0.636	3.723	0.155	18.974	0.791
30	28	12.621	0.451	5.319	0.190	17.940	0.641
	32	18.407	0.575	5.756	0.180	24.163	0.755
	36	16.304	0.453	6.383	0.177	22.687	0.630
	40	17.355	0.434	7.446	0.186	24.801	0.620
	44	19.458	0.442	6.833	0.155	26.291	0.598
	48	17.355	0.362	9.575	0.200	26.930	0.561
Average			0.575		0.238		0.813
	4	4.659	1.165	4.437	1.109	9.096	2.274
	8	4.659	0.582	4.437	0.555	9.096	1.137
	12	7.764	0.647	10.871	0.906	18.635	1.553
	16	9.096	0.569	14.199	0.888	23.295	1.456
	20	8.652	0.433	18.636	0.932	27.288	1.364
	24	9.540	0.398	17.748	0.740	27.288	1.137
54	28	8.874	0.317	19.079	0.681	27.953	0.998
	32	8.652	0.270	29.507	0.922	38.159	1.193
	36	8.430	0.234	29.951	0.832	38.381	1.066
	40	8.430	0.211	31.725	0.793	40.155	1.004
	44	8.208	0.187	33.572	0.763	41.780	0.950
	48	8.600	0.179	36.162	0.753	44.762	0.933
Average			0.432		0.824		1.255

TABLE 2—*Continued*

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		Ammonia per culture		Nitrate per culture		Total nitrogen per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
66	4	0.920	0.230	7.584	1.896	8.504	2.126
	8	1.608	0.201	15.167	1.896	16.775	2.097
	12	1.608	0.134	23.439	1.953	25.043	2.087
	16	2.298	0.144	32.172	2.011	34.470	2.154
	20	3.447	0.172	34.470	1.724	37.917	1.896
	24	3.447	0.144	39.986	1.666	43.433	1.810
	28	5.055	0.181	41.364	1.477	46.419	1.658
	32	5.745	0.180	48.258	1.508	54.003	1.688
	36	5.975	0.166	48.800	1.356	54.775	1.522
	40	11.490	0.287	47.407	1.185	58.897	1.473
	44	12.869	0.293	55.382	1.259	68.251	1.511
	48	13.329	0.278	64.344	1.341	77.673	1.618
Average			0.201		1.606		1.807
78	4	2.933	0.733	7.332	1.833	10.265	2.566
	8	2.933	0.366	9.971	1.246	12.904	1.612
	12	2.933	0.244	17.595	1.466	20.528	1.710
	16	2.933	0.183	17.595	1.100	20.528	1.283
	20	5.279	0.264	17.595	0.880	22.874	1.144
	24	5.573	0.232	25.062	1.044	30.635	1.276
	28	2.933	0.105	33.431	1.194	36.364	1.299
	32	2.933	0.092	40.032	1.251	42.965	1.343
	36	5.573	0.155	38.123	1.059	43.696	1.214
	40	8.798	0.183	52.785	1.320	61.583	1.503
	44	9.384	0.213	54.252	1.233	63.636	1.446
	48	11.144	0.232	61.583	1.283	72.727	1.515
Average			0.250		1.242		1.493
90	4	1.596	0.399	4.106	1.027	5.702	1.426
	8	3.069	0.384	7.038	0.880	10.107	1.263
	12	1.596	0.133	9.971	0.831	11.567	0.964
	16	2.346	0.147	11.730	0.733	14.076	0.880
	20	2.639	0.132	11.730	0.587	14.369	0.719
	24	2.346	0.198	21.114	0.880	23.460	0.978
	28	2.054	0.073	32.408	1.157	34.462	1.231
	32	1.467	0.046	39.590	1.237	41.057	1.283
	36			35.190	0.978	35.190	0.978
	40	2.625	0.066	45.161	1.129	47.786	1.195
	44	0.294	0.007	45.455	1.033	45.749	1.040
	48	2.640	0.055	52.785	1.100	55.425	1.155
Average			0.128		0.964		1.092

TABLE 2—*Concluded*

AGE OF PLANTS	ABSORPTION INTERVAL	QUANTITY OF NITROGEN ABSORBED AS					
		Ammonia per culture		Nitrate per culture		Total nitrogen per culture	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	4	1.331	0.333	1.775	0.444	2.506	0.777
	8	1.553	0.194	4.881	0.610	6.434	0.804
	12	1.553	0.129	8.438	0.703	9.991	0.833
	16	1.331	0.083	11.537	0.721	12.868	0.804
	20	1.997	0.100	15.752	0.788	17.749	0.888
102	24	4.437	0.185	15.530	0.647	19.967	0.832
	28	4.437	0.159	17.526	0.626	21.963	0.784
	32	4.437	0.139	23.295	0.728	27.732	0.867
	36	3.993	0.111	23.960	0.666	27.953	0.777
	40	3.771	0.094	22.185	0.555	25.956	0.649
	44	2.219	0.050	22.851	0.519	25.070	0.570
	48	2.441	0.051	23.295	0.485	25.736	0.536
Average			0.136		0.624		0.760

plants with respect to nitrogen intake at the different stages of development in the life cycle. The results obtained by the two methods of calculation will be presented and considered separately.

Absorption of nitrogen per culture per hour (*quantity absorption*). The data dealing with the absolute quantities of nitrogen absorbed in the two forms, in milligrams per culture per hour, are presented in table 2. The data in this table are arranged in precisely the same manner as are those in table 1. As in the preceding table, each of the values except those marked "average," represents the average of four determinations. Each of the values marked "average" in the table represents the final average value of 48 determinations involving 24 cultures and 72 plants. These final average values only will be considered. They were plotted to form the graphs of figure 4.

It will be observed that the graph representing the average quantity absorption of nitrogen as NH_4 per culture per hour shows a gradual decrease in values for the experimental stages from the first stage at 18 days to the last at 102 days. It indicates during the early stages of growth a high quantity absorption of nitrogen as NH_4 per culture per hour which gradually decreases with the age of the plant. It is thus apparent that the maximum absorption of nitrogen as ammonia per culture per hour occurs at a very early stage of development and while the plants are still very young and small, in spite of the fact that the size, weight, and area of absorbing surface of the plant rapidly increase with age. During the later stages of growth the quantity intake of nitrogen as NH_4 becomes very low but at no time during the active life cycle does the plant entirely cease to absorb nitrogen as NH_4.

On the other hand, the graph representing the average quantity absorption of nitrogen as NO_3 per culture per hour begins with very low values and rises rapidly with increase in size, weight, and area of absorbing surface as the plants become older, reaching a maximum point at the 66-day stage. Following this, the graph takes a steep downward pitch, the average values decreasing with the age of the plant from this point. Thus during the early stages of growth a low average NO_3 absorption per culture per hour is indicated,

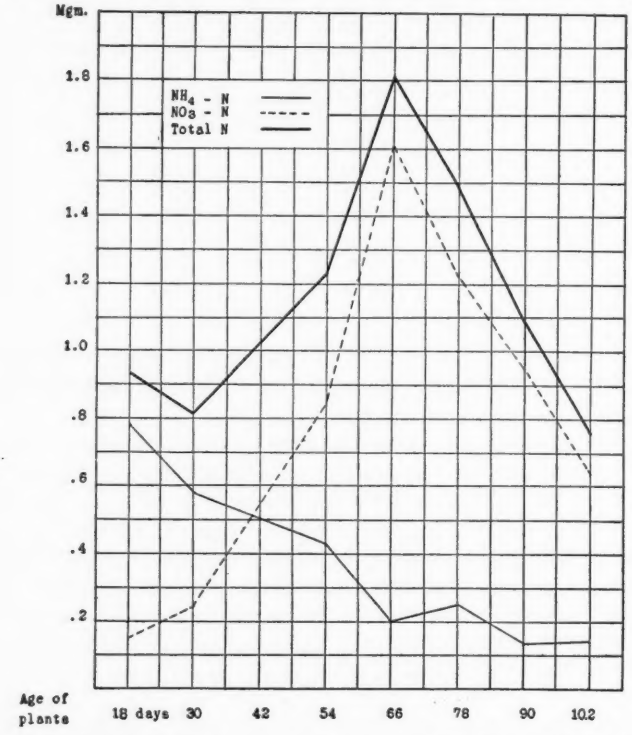

FIG. 4. GRAPHS REPRESENTING QUANTITY OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN ABSORBED, IN MILLIGRAMS PER CULTURE PER HOUR AT DIFFERENT PERIODS THROUGHOUT THE LIFE CYCLE OF THE OAT PLANT

with rapidly increasing values up to and including the reproductive phase at 66 days, after which the absorption values decrease rapidly with the age of the plants, as indicated by the steep downward pitch of the graph.

The graph representing average total nitrogen absorption per culture per hour in figure 4 first takes a downward slope between the 18-day and the 30-day period. The graph thus indicates a lower quantity absorption of total nitrogen when the plants were 30 days old than when they were only 18 days

old. This lower average intake at 30 days is directly correlated with a period of low light intensity, since the tests were made at this time during a period of cloudy weather and high humidity. This is an indication that external conditions, particularly conditions of light intensity, have a pronounced influence upon the absorption of nitrogen by the plants. The graph takes a steep upward slope from the point representing the 30-day period to that representing the 66-day period, the latter occurring during the time of flowering. At this point the maximum quantity absorption of total nitrogen per culture per hour is reached. This corresponds also to the point indicating the maximum absorption of nitrogen as nitrate. After the maximum point is reached, a steep downward slope is taken by the graph, indicating the rapid decrease in total nitrogen absorption per culture per hour during the late stages of growth and maturity. It is thus clear that the course of this graph representing the early stages of growth is determined mainly by quantity absorption of nitrogen as NH_4, whereas during the period of blossoming and later development the course of the graph is determined by quantity absorption of nitrogen as NO_3. The graphs of figure 4 very clearly bring out the fact that in a medium in which nitrogen is simultaneously present in the forms of ammonium and nitrate in approximately equal proportions, the plants while young satisfy the nitrogen requirement mainly through the absorption of ammonium, whereas during the later stages of development the plants obtain their nitrogen largely through the absorption of nitrate. However, at no time during the active growth cycle is the absorption of nitrogen either as ammonium or as nitrate completely at a standstill under the conditions of these experiments.

Absorption of nitrogen per unit of dry plant material per hour (rate absorption). The data dealing with the actual rates of absorption of nitrogen in the different forms calculated on the basis of intake per gram of dry plant material per hour are presented in table 3. As before, only the final average values as given in the table will here be considered. For the sake of convenience, the values representing nitrogen absorbed as NH_4, in milligrams per gram of dry plant material per hour will be designated the NH_4-rate, and the values representing nitrogen absorbed as NO_3 in milligrams per gram of dry plant material per hour will be designated the NO_3-rate. The final average rate values as given in table 3, were plotted to form the graphs of figure 5.

It will be observed that the course of the graph representing NH_4-rates of absorption per gram of dry plant tissue per hour shows a very steep downward slope at first and then a more gradual downward slope from the 30-day period to the 66-day period, and shows very low rates during the later phases of growth. Thus the graph indicates exceptionally high NH_4-rates during the early growth phases and very low NH_4-rates during the later phases of growth from the flowering period to maturity.

On the other hand, the graph representing the NO_3-rates, per gram of dry plant material per hour, shows very low rates at first which gradually rise

TABLE 3

Absorption of nitrogen as NH_4, NO_3, and total nitrogen per gram of dry plant tissue by oats during absorption intervals ranging in time from 4 hours to 48 hours at different stages of development throughout the life cycle

AGE OF PLANTS	ABSORPTION INTERVALS	DRY WEIGHT PER CULTURE	ACTUAL NITROGEN ABSORBED—MGM. PER GRAM DRY WEIGHT PER HOUR		
			NH_4–N	NO_3–N	Total N
days	*hours*	*gm.*			
	4	0.587	1.567	0.226	1.794
	8	0.583	1.466	0.342	1.808
	12	0.599	1.464		1.464
	16	0.595	1.380	0.391	1.771
	20	0.586	1.391	0.272	1.663
18	24	0.576	1.332	0.231	1.563
	28	0.565	1.197	0.303	1.500
	32	0.573	1.206	0.203	1.409
	36	0.584	1.251	0.253	1.504
	40	0.573	1.262	0.232	1.494
	44	0.578	1.283	0.335	1.618
	48	0.582	1.298	0.361	1.660
Average		0.582	1.341	0.262	1.604
	4	1.224	0.645	0.326	0.971
	8	1.575	0.626	0.246	0.872
	12	1.414	0.496	0.251	0.747
	16	1.210	0.408	0.188	0.595
	20	1.405	0.412	0.170	0.582
	24	1.436	0.443	0.108	0.551
30	28	1.201	0.475	0.158	0.534
	32	1.486	0.387	0.121	0.508
	36	1.497	0.303	1.118	0.421
	40	1.398	0.310	0.133	0.444
	44	1.475	0.300	0.105	0.405
	48	1.246	0.390	0.160	0.550
Average		1.381	0.416	0.174	0.590
	4	2.432	0.479	0.456	0.935
	8	2.310	0.252	0.240	0.492
	12	2.294	0.282	0.395	0.677
	16	2.257	0.252	0.393	0.645
	20	2.453	0.176	0.380	0.556
	24	2.101	0.189	0.352	0.541
54	28	2.014	0.157	0.338	0.496
	32	2.219	0.122	0.416	0.537
	36	2.240	0.105	0.371	0.476
	40	2.306	0.091	0.344	0.435
	44	2.315	0.081	0.330	0.410
	48	2.194	0.082	0.343	0.425
Average		2.261	0.189	0.363	0.552

TABLE 3—*Continued*

AGE OF PLANTS	ABSORPTION INTERVALS	DRY WEIGHT PER CULTURE	ACTUAL NITROGEN ABSORBED—MGM. PER GRAM DRY WEIGHT PER HOUR		
			NH_4–N	NO_3–N	Total N
days	*hours*	*gm.*			
	4	2.843	0.081	0.669	0.750
	8	2.792	0.072	0.679	0.751
	12	2.833	0.047	0.689	0.737
	16	2.714	0.053	0.741	0.794
	20	2.649	0.065	0.651	0.716
66	24	2.704	0.053	0.616	0.667
	28	2.640	0.068	0.560	0.628
	32	2.520	0.071	0.599	0.670
	36	2.510	0.066	0.540	0.606
	40	2.772	0.036	0.428	0.531
	44	2.790	0.048	0.451	0.556
	48	2.508	0.107	0.535	0.645
Average		2.690	0.075	0.596	0.671
	4	3.153	0.233	0.581	0.814
	8	3.082	0.119	0.404	0.523
	12	3.178	0.077	0.461	0.538
	16	3.052	0.060	0.360	0.421
	20	2.660	0.099	0.331	0.430
	24	3.004	0.077	0.348	0.425
78	28	3.024	0.035	0.395	0.430
	32	3.012	0.030	0.415	0.446
	36	2.940	0.053	0.360	0.413
	40	3.120	0.059	0.423	0.482
	44	3.080	0.069	0.400	0.470
	48	3.193	0.073	0.402	0.475
Average		3.042	0.082	0.407	0.489
	4	3.420	0.117	0.300	0.417
	8	3.396	0.113	0.259	0.372
	12	3.384	0.039	0.246	0.285
	16	3.322	0.044	0.221	0.265
	20	3.275	0.043	0.179	0.219
	24	3.320	0.030	0.264	0.294
90	28	3.298	0.023	0.251	0.374
	32	3.495	0.013	0.354	0.367
	36	3.341		0.292	0.292
	40	3.431	0.019	0.329	0.348
	44	3.358	0.002	0.308	0.310
	48	3.409	0.016	0.323	0.339
Average		3.371	0.038	0.285	0.323

TABLE 3—*Concluded*

AGE OF PLANTS	ABSORPTION INTERVALS	DRY WEIGHT PER CULTURE	ACTUAL NITROGEN ABSORBED—MGM. PER GRAM DRY WEIGHT PER HOUR		
			NH_4–N	NO_3–N	Total N
days	*hours*	*gm.*			
	4	4.103	0.081	0.108	0.189
	8	4.260	0.046	0.143	0.188
	12	4.295	0.030	0.133	0.163
	16	4.194	0.020	0.172	0.192
	20	4.302	0.023	0.183	0.206
102	24	4.148	0.045	0.156	0.201
	28	4.086	0.039	0.153	0.129
	32	4.242	0.033	0.172	0.204
	36	4.160	0.027	0.160	0.187
	40	4.156	0.023	0.133	0.156
	44	4.123	0.012	0.126	0.138
	48	4.034	0.013	0.120	0.133
Average		4.175	0.032	0.147	0.179

with increase in the age of the plant, reaching a maximum point at the 66-day experimental period, or the flowering stage of the plants. Thereafter the NO_3-rates decrease in value as the plants approach maturity. Thus during the early stages of growth, low NO_3-rates of absorption are indicated for the oat plant, relatively high rates during the reproductive phase, with declining rates from the blossoming stage to maturity.

The graph representing rates of total nitrogen absorption takes a very steep downward slope from the point representing the 18-day period to that representing the 30-day period, with a secondary maximum point at the 66-day period. The graph then shows continuously declining rates as the plants approach maturity. The secondary maximum point in the graph of total nitrogen absorption rates is the result of the high NO_3-rates at this point.

A consideration of these rate graphs makes it clear that the rates of nitrogen absorption by the young plants are completely dominated by the NH_4-rates, whereas from the blossoming period to maturity nitrogen absorption is dominated by the NO_3-rates, under the conditions of these experiments when the two forms of nitrogen are present in the external medium in approximately equal proportions. The graphs further show that the maximum NH_4-rate, which occurs in the very young plants, is more than double the maximum NO_3-rate, which occurs during the blossoming period. There are thus indicated two periods in the life cycle when the plants show higher activity with respect to nitrogen absorption than they do at other periods: the highest activity occurring at a very early stage, and the second period of high activity occurring during the blossoming stage.

A comparison of the graphs of figure 2 with those of figure 3 brings out the fact that the maximum total quantity of nitrogen absorbed per culture in unit

time occurs at the same point in the life cycle (blossoming period) as does the maximum NO_3-rate but this does not represent the maximum rate of total nitrogen absorption, which, as indicated by the rate graphs of figure 3, occurs at the earliest period in the life cycle here investigated and corresponds with the occurrence of the maximum NH_4-rate.

It is, of course, to be emphasized in this connection that the results here obtained apply only to the oat plant grown in a culture solution in which the nitrogen is simultaneously present in the two forms in approximately equal

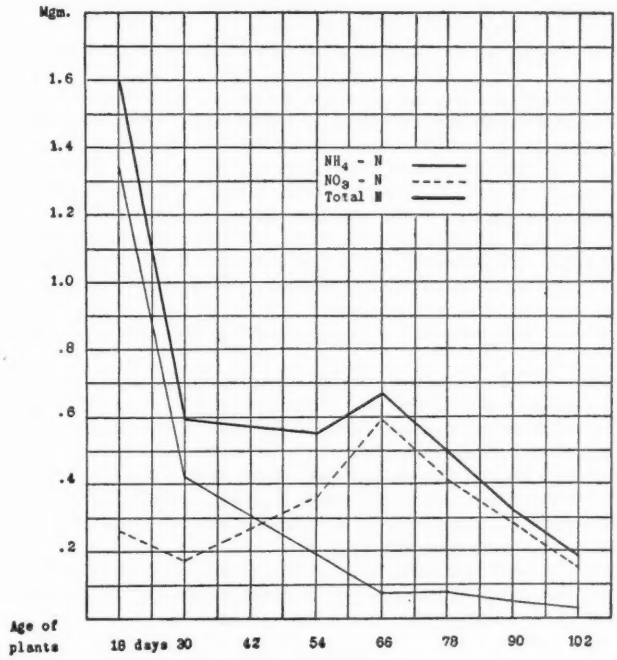

FIG. 5. GRAPHS REPRESENTING THE RATES OF ABSORPTION OF NITROGEN AS NH_4, NO_3 AND TOTAL NITROGEN, IN MILLIGRAMS PER GRAM OF DRY PLANT MATERIAL PER HOUR, AT DIFFERENT PERIODS THROUGHOUT THE LIFE CYCLE OF THE OAT PLANT

proportions. However, in the preliminary tests here reported, approximately the same results were obtained with a solution in which the nitrogen in the two forms is present in quite unequal proportions. No consideration has here been given to the influence of the proportions of the non-nitrogen bearing salts or ions in the culture solution on the rates of nitrogen absorption. What would be the influence of widely different chemical and physical properties of culture media, or widely different sets of environmental conditions, upon the rates of nitrogen absorption from culture solutions, cannot now be stated.

SUMMARY

Quantitative studies were made of the absorption of nitrogen by oat plants from culture solutions containing approximately equal proportions of nitrogen as NH_4 and NO_3, at various stages in the life cycle of the plants. The main results of the investigation may be summarized briefly as follows:

Quantitative analyses show that quantity absorption of nitrogen as NH_4 (milligrams per culture per hour) is highest during the earliest period here investigated, gradually declines, and reaches its minimum point at maturity.

Quantity absorption of nitrogen as NO_3 (milligrams per culture per hour) shows its minimum point at the earliest period investigated, rapidly increases, and reaches a maximum at the blossoming stage, and then rapidly declines to a secondary minimum with approaching maturity.

Quantity absorption of total nitrogen attains a maximum point which corresponds to the point of maximum absorption of nitrogen as NO_3. This occurs during the flowering period.

At no time during the active life cycle was it found that the absorption of nitrogen as NH_4 or as NO_3 entirely ceased.

Rate absorption of nitrogen as NH_4 (milligrams per gram of dry tissue per hour) is at its maximum during the earliest period here investigated, declines rapidly during the early stages of growth, and reaches a very low minimum rate with approaching maturity.

Rate absorption of nitrogen as NO_3 (milligrams per gram of dry tissue per hour) is low during the early stages of development, attains its maximum at the blossoming stage, then declines to a secondary minimum at maturity.

The maximum NH_4-rate of absorption of nitrogen is more than double the maximum NO_3-rate.

Rate absorption of total nitrogen attains a maximum point which corresponds to the point of the maximum NH_4-rate of nitrogen absorption and is determined by the NH_4-rate. A secondary maximum rate occurs at the blossoming stage, corresponds to, and is determined by, the point of the maximum NO_3-rate.

Two periods in the growth cycle occur when the plants show higher activity with respect to the rate of nitrogen absorption (milligrams per gram dry plant material per hour) than they do at other periods: the greatest activity occurring at a very early stage and the second period of high activity during the blossoming stage.

Data are presented to emphasize the importance of some adequate system of continuous flow solution renewal, not only in nutritional studies with plants in culture solutions, but also for the growth of experimental plants in solution or sand cultures.

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LYSIMETER STUDIES: III. THE MOVEMENT AND TRANSLOCATION OF NITROGEN AND ORGANIC CONSTITUENTS IN THE PROFILE OF A PODZOLIC SOIL[1]

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The organic matter and the nitrogen of the soil are intimately related, inasmuch as most of the combined nitrogen, irrespective of the source, is tied up with the organic matter. It is the all important constituent in the nitrogen cycle in nature. It serves as the potential storehouse and accumulator—directly as the organic nitrogenous compounds and indirectly as a source of energy for the microbial flora involved in the process of nitrogen fixation—of the soil's nitrogen resources.

One of the important links in the nitrogen cycle is the process of nitrification with nitrates as the end product. Nitrates are the most mobile nitrogenous constituents and in the balance sheet of nitrogen economy in the soil are very prominent. In the studies on the nitrogen cycle in the soil the nitrates have received the lion's share of attention.

Volumes have been written reporting the investigations on the nitrogen cycle in the soil. A cursory survey of the research projects in the various experiment stations in the United States and elsewhere shows that the nitrogen problem occupies an important place in the research programs of these institutions. Our own, New Jersey Experiment Station, widely known cylinder and plot experiments have been established with the primary view of studying the nitrogen problem, principally one phase of it—denitrification.

And yet this problem is still somewhat of a *terra incognito* and additional information is highly desirable. This is especially true now when our ideas about soils are undergoing a radical change and all soil reactions are being examined in the light of the newer knowledge about the soil profile.

The new lysimeter equipment installed at this station (2) offers an opportunity to study the movement and translocation of organic matter and nitrogen through the undisturbed soil profile. The results which are presented in the following pages seem to give some hitherto unknown and, in a way, unexpected facts.

PERCOLATION OF NITROGEN THROUGH PROFILE

For cropped land, it is generally accepted that the nitrogen losses from the soil in the drainage consist primarily of nitrates. According to Lyon and

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

Bizzell (5, p. 61) "nitrogen in the drainage water is *all* in the form of nitrates." Collison and Mensching (1, p. 40) report that "*almost all* of the nitrogen in the drainage water is in the nitrate form." We shall see presently that this does not, necessarily, hold true for virgin soils.

According to Lyon, Bizzell, Wilson, and Leland (6), the average annual loss of nitrogen in the drainage is 75 pounds for the unplanted tank without lime and 61 pounds for the unplanted tank with lime. Losses of nitrogen have been found to be about 9 to 10 times less from the cropped tanks. The tanks which had a grass crop lost the least amount of nitrogen. A complicating factor in the interpretation of the Cornell lysimeter results is the mode of treatment, namely, the tanks received manure applications which for the period recorded added to the soil 564 pounds of nitrogen per acre. If we assume that all of the nitrogen from the manure disappeared in the drainage as nitrates—an assumption which is unjustified—there is an equivalent of about half of the nitrogen noted above lost from the soil proper.

Collison and Mensching (1) in reporting the results of the lysimeter investigations at the Geneva Experiment Station give the figure of 120.6 pounds of nitrogen per acre lost annually in the drainage from the fallow tanks.

Miller (8) in reporting the results of the lysimeter investigations at Rothamsted gives the figure on the average annual loss of nitrogen from the fallow tank as 30 pounds per acre of which 27 pounds is in the form of nitrates. These figures were substantiated later by Russell and Richards (9).

Many reports of a similar nature on the losses of nitrogen in drainage may be found in the literature concerning lysimeter investigations in Germany and elsewhere, but the three mentioned may suffice to illustrate the difficulties which we encounter when we attempt a correct appraisal of the losses of nitrogen in drainage. Whereas the three stations mentioned show wide variations in their reports on the nitrogen losses, these may not be attributed to differences in soils and meteorological conditions. This is especially true for the Cornell and Geneva stations, where the drainage has been measured from soil material variously prepared and not from the soil body, as pointed out in a former publication (2).

The Rothamsted results are more reliable inasmuch as the soil itself has been preserved in its natural state. Otherwise the conditions have remained similar to those of the other lysimeter installations: the soil is separated from the bulk of the soil body by the tank inclosure, the rainwater must go through the soil column without any chance for return capillary movement from the ground waters and there is no surface run-off.

Tables 1 and 2 present the results on the total and nitrate nitrogen in the drainage obtained from the A_1 horizon for two consecutive years by the new type of lysimeters[2] whereby the conditions are very close to natural.

The first to be noted is that not all of the nitrogen is in the nitrate form.

[2] A full description of the new type of lysimeter equipment is to be found in an earlier publication (2).

In 1929–30 only 74.6 per cent and in 1930–31 about 60 per cent were in the form of nitrates. The rest of the nitrogen is in some other forms. Very little of the nitrogen seems to appear as ammonia, although qualitatively its presence could be demonstrated. For the present the nitrogen besides the nitrates is to be considered as in the organic form.

TABLE 1

Total N and NO_3N percolated through A_1 in 1929–30

(Leachings for year = 21 liters)

LABORATORY NUMBER OF LEACHING ON WHICH DETERMINATIONS WERE MADE	DATE OF COLLECTION	QUANTITY COLLECTED	TOTAL N IN		NO_3–N IN	
			100 cc. of leachings	Total leachings	100 cc. of leachings	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1929					
3	7/30	1.400	0.631	8.831	0.22	3.080
4	8/15	0.755	0.760	5.738		
5	9/9	4.740	0.640	30.336	0.48	22.752
7	10/3	3.440	0.650	22.360	0.50	17.200
8	10/23	0.930	0.660	6.138	0.48	4.460
9	11/5	0.390			0.48	1.870
11	12/20	0.180			0.48	5.660
	1930					
12	2/18	3.435	0.252	8.656	0.16	5.500
15	6/11	2.200	0.310	6.820	0.24	5.280
1. Total			3.903	88.879	3.04	65.802
2. Average per 100 cc.*			0.558	0.526	0.380	0.394
3. Calculated totals†				110.46		82.74
4. Total per acre in grams‡				6,075.3		4,550.7
5. Total per acre in pounds				13.4		10.0

* The average per 100 cc. under the columns "total leachings" was calculated by dividing the total constituents by the respective total leachings on which determinations were made; in the case of total N by 16.9 liters, and in the case of NO_3 by 16.715 liters.

† To obtain the total for all the leachings the average per 100 cc. as calculated was multiplied by the total leachings for the year, 21.0 liters.

‡ To obtain grams per acre the figures from 3 were multiplied by 55,000, since the area of the lysimeter funnel = $\frac{1}{55,000}$ of an acre, and divided by 1,000 (1 gm. = 1,000 mgm.).

The source of the organic form of nitrogen is the A_0 layer which supplies yearly large quantities of organic matter for the process of decomposition and mineralization. In cultivated soils we find the organic matter in the form of stubble, roots, and weed growth, most of which, because of favorable conditions for decomposition, is completely mineralized. This will, perhaps, explain why only 10 per cent of the total nitrogen was not in the form of nitrates in the Rothamsted experiments. Why there was no other form of

nitrogen in the Cornell lysimeters, where large quantities of organic matter in the form of manure were added periodically, is difficult to explain.

Seasonal variations in concentration of nitrogen in the leachings are not so clearly brought out in the data. There is a tendency for a higher concentration of nitrogen compounds toward the end of the summer and in the fall. This is in line with the findings about the mineral constituents in the leachings as brought out in a previous publication (3). The concentration of ni-

TABLE 2

Total N and NO_3 N percolated through A_1 in 1930–31

(Leachings for year = 29.223 liters)

LABORATORY NUMBER OF LEACHING ON WHICH DETERMINATIONS WERE MADE	DATE OF COLLECTION	QUANTITY COLLECTED	TOTAL N IN		NO_3–N IN	
			100 cc. of leachings	Total leachings	100 cc. of leachings	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1930					
16	7/10	1.300	0.423	5.502	0.240	3.120
17	7/23	1.300	0.480	6.240	0.120	1.560
19	8/24	3.350	0.600	20.100	0.140	4.690
23	11/6	1.455	0.693	10.083		
24	11/19	1.875	0.839	15.728		
25	12/30	4.090	0.651	26.626	0.540	22.086
	1931					
26	2/19	4.100	0.353	14.473	0.280	11.480
27	3/9	1.770	0.620	10.974	0.440	7.788
28	3/29	3.305	0.466	15.378	0.360	11.880
29	4/2	0.665			0.240	1.596
30	4/12	0.505			0.180	0.909
33	6/11	1.950	0.480	9.360		
1. Total			5.605	134.464	2.54	65.109
2. Average per 100 cc.*			0.5605	0.549	0.282	0.319
3. Calculated totals†				160.43		93.22
4. Total per acre in grams‡				8,823.7		5,127.1
5. Total per acre in pounds				19.4		11.3

* † ‡ For explanations of footnotes see table 1.

trogen compounds does not seem to decrease much with dilution during any one season. With respect to the nitrates the concentration factor seems to follow the trend of the total nitrogen, although some departures might be pointed out. More data are wanted before anything definite may be said about this point.

Tables 3 and 4 give the data on the total nitrogen and nitrates which percolated through the A_2 horizon, which is located—the bottom of it—at a depth of 42 cm. in the profile.

TABLE 3

Total N and NO_3 percolated through A_2 in 1929–30

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	QUANTITY COLLECTED	TOTAL N IN		NO_3—N IN	
			100 cc. of leachings	Total leachings	100 cc. of leachings	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1929					
1	6/22	0.165				
5	9/9	2.400	0.482	11.568	0.16	3.840
7	10/3	0.210				
11	12/20	0.090				
	1930					
12	2/18	1.265	0.504	6.376	0.32	4.048
15	6/11	1.395			0.40	5.580
1. Total		5.525	0.986	17.944	0.88	13.468
2. Average per 100 cc.*			0.493	0.484	0.293	0.266
3. Calculated totals†			26.74		14.69	
4. Total per acre in grams‡					808.00	
5. Total per acre in pounds			3.24		1.78	

* † ‡ For explanations on footnotes see table 1.

TABLE 4

Total N and NO_3 percolated through A_2 in 1930–31

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	QUANTITY COLLECTED	TOTAL N IN		NO_3—N IN	
			100 cc. of leachings	Total leachings	100 cc. of leachings	Total leachings
		liters	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	1930					
25	12/30	1.870	0.750	14.025	0.45	8.415
	1931					
26	2/19	1.200	0.366	4.392	0.16	1.920
27	3/9	0.250			0.32	0.800
28	3/30	0.910			0.18	1.638
34	6/18	0.045				
1. Total		4.275	1.116	18.417	1.11	12.773
2. Average per 100 cc.*			0.558	0.600	0.28	0.302
3. Calculated totals†			25.55		12.91	
4. Total per acre in grams‡			1,405.3		710.1	
5. Total per acre in pounds			3.09		1.56	

* † ‡ For explanations on footnotes see table 1.

The first to be noted is that out of 13.4 pounds of nitrogen which leached through A_1 in 1929–30 only 3.24 pounds, or 24.1 per cent, percolated through A_2. It will be recalled (2) that in the corresponding year the leachings from A_2 amounted to 25 per cent of those from A_1. Of the 19.4 pounds of nitrogen which leached through A_1 in 1930–31 only 3.09 pounds, or 15.9 per cent, percolated through A_2. And during this year the leachings from A_2 amounted to 14.6 per cent of those from A_1. Apparently there is a certain correlation between the total nitrogen compounds leached from A_2 and the total leachings.

Another point to be noted in the data on the nitrogen percolation through A_2 is that in both years the amount of nitrate nitrogen with respect to the totals is about the same: a little over 50 per cent. If we compare the forms of nitrogen in the A_1 and A_2 we note that the nitrate nitrogen drops in A_2. Naturally this means that the other forms of nitrogen, especially organic, are on the increase.

The decrease in nitrates in the leachings of A_2 is readily accounted for. During the summer very few leachings appear from A_2; they are retained there and utilized by the growing plants. Most of the leachings from A_2 appear in the winter when the nitrate content drops as a result of the slowing down of the nitrification process.

The data on the leachings from A_2 are scant because there are usually not enough leachings to make total nitrogen analyses. More data are wanted to corroborate the conclusions presented in the foregoing discussion.

ORGANIC MATTER AND NITROGEN RELATIONSHIPS IN LEACHINGS

The movement of organic matter in the soil profile is of paramount importance in elucidating the processes of soil formation. It is generally recognized that it is the organic matter that is responsible for the movement of the sesquioxides and other mineral constituents, especially in the podzols.

The data on the total solids, loss on ignition, and the estimated 10 percent water of hydration (this figure is only an approximation, as it was calculated from the probable water of hydration in the mineral residue) furnish the information on the organic matter in the leachings.

In tables 5 and 6 the data on the total solids and loss on ignition are confined only to the leachings on which nitrogen determinations were made. A complete record of these was presented in a former publication (3). It was found that in 1929–30 the loss on ignition, calculated on the acre basis, was 139.6 pounds, or 125.64 pounds of organic matter percolated through A_1. In 1930–31 the loss on ignition was 258.4 pounds, or 232.56 pounds of organic matter percolated through A_1. For the corresponding years the organic matter in the leachings of A_2 was 19.69 and 13.32 pounds respectively. Thus more organic matter was leached from A_2 in 1929–30 than in 1930–31, notwithstanding the fact that less organic matter reached it from A_1. The converse is true for the organic matter from A_2 in 1930–31.

An examination of the quantities of leachings from A_2 for the 2 years under

consideration shows that in 1929–30 more appeared than in 1930–31 —5.525 and 4.275 liters respectively—even though a smaller quantity of leachings reached A_2 from A_1 in that year—21 in 1929–30 against 29.2 liters in 1930–31.

TABLE 5

Carbon and nitrogen relationships in leachings from A_1

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	LEACHINGS	TOTAL SOLIDS AT 105°C.	LOSS ON IGNITION		TOTAL CARBON*	ORGANIC N†	C:N RATIO	N IN ORGANIC MATTER‖
		liters	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*		*per cent*
				1929–30					
	1929								
3	7/30	1.400	289.80	142.24	49.00	74.24	5.751	12.9	4.5
4	8/15	0.755	113.25	43.79	38.6	22.86	2.866‡	8.0	7.4
5	9/9	4.740	611.46	271.13	44.3	141.53	7.584	18.6	3.1
7	10/3	3.440	509.80	181.97	35.6	94.99	5.160	18.4	3.1
8	10/23	0.930	189.72	66.96	35.2	34.95	1.678	20.8	2.8
	1930								
12	2/18	3.435	378.54	78.49	20.7	40.97	3.156	13.0	4.4
15	6/11	2.200	300.96	133.32	44.2	69.59	1.540	45.0	1.3
				1930–31					
	1930								
16	7/10	1.300	245.70	161.42	65.6	84.26	2.38	35.4	1.6
17	7/23	1.300	158.60	91.78	57.8	47.91	4.68	10.2	5.6
19	8/24	3.350	739.68	404.68	54.7	211.24	15.41	13.7	4.2
23	11/6	1.455	480.73	218.25	45.3	113.93	5.44†	20.9	2.7
24	11/19	1.875	571.12	190.69	33.3	99.54	9.75†	10.2	5.6
25	12/30	4.090	503.07	204.45	40.6	106.73	4.54	23.5	2.4
	1931								
26	2/19	4.100	434.60	169.74	39.0	88.61	2.99	29.6	1.9
27	3/9	1.770	180.19	64.61	35.8	33.73	3.19	10.6	5.5
28	3/29	3.305	311.33	115.68	37.1	60.38	3.50	17.2	3.4
33	6/11	1.950	308.10	187.20	60.7	97.72	3.14	31.1	1.8

* The figures on the total carbon were obtained as follows: On the total ignition loss of solids 10 per cent was allowed for water of hydration and the rest was considered as organic matter. By taking the generally accepted 58 per cent of C in organic matter, the total C was calculated.

† Organic N was calculated by subtracting the NO_3–N from the figures on the total N.

‡ The NO_3–N on this sample was calculated by taking the average NO_3 for the year.

‖ The nitrogen in organic matter was calculated as follows: The organic matter was figured out as shown in footnote* and from the[illegible] and the figures on the organic N the per cent of N in the organic matter was obtained.

The explanation for this phenomenon was discussed in the first paper of this series (2). It is the state of the colloids in the soil that controls the percolation rather than the total volume of rainfall or leachings.

The average percentage loss on ignition of the solids from A_1 was higher in 1930–31 than in 1929–30, about 46 and 38 per cent respectively. This, of course, is due to the higher organic matter content in the leachings of 1930–31. As a matter of fact, the concentration of organic matter was also higher, 15.9 and 14.21 mgm. of solids per 100 cc. of leachings in 1930–31 and 1929–30 respectively. The cause of such a higher concentration is, of course, difficult to explain. Apparently the environmental conditions for microbial activities in the A_1 horizon were more favorable in 1930–31 than in 1929–30. Unfortunately practically nothing has been done on the differential behavior of microörganisms in the various horizons of the soil profile.

TABLE 6

Carbon and nitrogen relationships in leachings from A_2

LABORATORY NUMBER OF LEACHING	DATE OF COLLECTION	LEACHINGS	TOTAL SOLIDS AT 105°C.	LOSS ON IGNITION		TOTAL CARBON*	ORGANIC N†	C:N RATIO	N IN ORGANIC MATTER
		liters	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*		*per cent*
				1929–30					
	1929								
5	9/9	2.400	242.40	69.60	28.7	36.33	7.728	4.7	12.3
	1930								
12	2/18	1.265	146.7	45.4	30.9	23.20	2.328	10.0	5.7
15	6/11	1.395	113.27	54.68	48.2	28.54	1.172†	24.3	2.4
				1930–31					
	1930								
25	12/30	1.870	204.20	71.06	34.3	37.09	5.610	6.6	8.7
	1931								
26	2/19	1.200	89.76	24.96	27.8	13.03	2.472	5.2	10.0
28	3/30	0.910	65.52	18.29	27.9	9.55	3.820	2.5	23.0

* † ‡ See footnotes in table 5.

The loss on ignition of the solids from A_2 was higher in 1929–30 than in 1930–31, 34.8 and 31.7 per cent respectively. [These figures are taken from the second paper of this series (3) where all the leachings are recorded.] Again the higher organic matter content is responsible for the higher loss on ignition. It is to be noted that the concentration of organic matter in the leachings of A_2 dropped considerably when compared with the leachings from A_1: 77 per cent in 1929–30 and 82 per cent in 1930–31.

There is considerable difference in the chemical make-up of the organic matter of the various horizons in the profile. This is very lucidly brought out in the data on the C:N ratios of the organic matter in the leachings from A_1 and A_2. During both years the ratio was high for the organic matter in the leachings from A_1 and low for A_2. But even within each horizon there is a

difference in the organic matter from the leachings from year to year. If we disregard the extremely high C:N ratios, especially leaching number 15—a procedure justified by tests with statistical methods—and average the others we find 15.3 and 18.5 for A_1 and 7.3 and 4.8 for A_2 respectively during the 2 years under consideration. A higher C:N ratio indicates a type of organic matter low in protein content, hence with a low ash content and high loss on ignition. This is what apparently happened with the organic matter in the leachings from A_1 in 1930–31 and from A_2 in 1929–30: the organic matter contained constituents of a lower protein nature than the organic matter in the leachings from A_1 in 1929–30 and from A_2 in 1930–31, and hence the difference in loss on ignition.

An examination of the nitrogen content of the organic matter, as presented in the data of tables 5 and 6, shows that it follows the loss on ignition: the higher the loss on ignition the lower the nitrogen content and vice versa. And this is to be noted: in A_2 the nitrogen content of the organic matter is by far greater than in A_1—4.2 and 3.5 for A_1 and 9 and 13.9 for A_2 in 1929–30 and 1930–31 respectively. Of course these wide differences in nitrogen content are due to differences in the type of organic matter.

The data on the organic matter in the leachings of the A_1 and A_2 horizons and on the nitrogen content of it offer a clue to the phenomenon noted in a different connection—in a study of the podzolization process (4)—namely, that the B horizon becomes enriched with organic matter of a high nitrogen content. The extremely high N content—in one case as much as 23 per cent—in the organic matter of the leachings from A_2 calls for an explanation. There is the probability that fresh animal excreta had been deposited at the time of that leaching. And we know that such highly nitrogenous compounds as urea are found in animal excreta. An almost similar amount of N—22 per cent—in humus substances of a podzol soil has been reported by Weis (10, p. 192).

GENERAL DISCUSSION

An important point which the foregoing pages attempted to bring out is that in forest soils the losses of nitrogen either in the form of nitrates or in any other form are not so great as those reported for cultivated soils. The lingering of the leachings in the soil profile and the relatively small quantities of leachings available for the ground waters, as pointed out in the first paper of this series (2), have indicated such a course. Actual analyses of the leachings show that even if we should consider that the nitrogen which passes the A_2 horizon finds its way to the ground waters (it is very probable that a good share of it remains long enough in the B horizon to be taken up by the plants), there are compensating factors which more than cancel the losses. One need not go into the aspect of nitrogen fixation, which is fairly well established, although no definite quantitative value may be assigned to this source. The nitrogen supplied by the rainfall alone is quite sufficient to offset the

losses by leaching. If it were not for the N dissolved in the rainwater and that fixed by microörganisms, where and how could the soil— I have in mind the virgin soils and those cultivated soils which reached a definite nitrogen minimum—maintain its nitrogen supply?

Analyses of rainwater show that very definite quantities of nitrogen are added annually to the soil through this medium. According to Miller (7), who reviewed the subject very thoroughly (125 references are given) up to his time, and Wilson (11), who reviewed the subject up to a later period, the annual average addition of nitrogen to the soil by rainfall does not fall below the figure of 5 pounds per acre. The data in tables 3 and 4 show that only a little more than 3 pounds of nitrogen per acre goes through the A_2 horizon, a figure below the amount supplied by the rainfall.

Data on the quantity of leachings from horizons B and C during these two years and on the number of times leachings appeared from these horizons are so scant that no definite conclusions can be reached. In all, only 1 per cent of the leachings passed through the B horizon and about 2 per cent through C. For an explanation of this anomalous phenomenon the reader is referred to the first paper of this series (2).

From the meager data on hand there is evidence that an appreciable amount of nitrates is retained in the B horizon. Thus leachings from C give, as a rule, a lower nitrate content than the leachings from any other horizon. The quantities of leachings obtained from B and C were not sufficient for nitrogen determinations, but from theoretical considerations and tests on the organic colloid fraction of a podzol soil, it seems (4) that in these horizons the colloids are highly dispersed and the nitrogen content of the organic matter is high.

An important element in the process of moisture percolation through the profile and in all the reactions accompanying it is, of course, the rainfall. It is to be recalled that during the 2 years under consideration it was below normal, and although the percolation is not entirely dependent on the total rainfall, as shown in the first paper of this series (2), it remains to be seen what the results will be when the rainfall shall be normal or above normal.

SUMMARY

A discussion on the data of lysimeter investigations, as exemplified by the Cornell, Geneva, and Rothamsted reports, on the nitrogen losses from soils is presented showing wide variations.

The data on the nitrogen in the leachings from the new type of lysimeters show that in forest soils the nitrogen losses are not entirely in the form of nitrates. Only 50 to 75 per cent of the nitrogen appears as nitrates through the A_1 horizon and still less through the A_2 horizon.

Only 3.24 pounds of total nitrogen percolated through A_2 in 1929–30 and only 3.09 pounds in 1930–31. Thus only small amounts of total nitrogen are lost.

Data on the organic matter content in the leachings show that the type

of organic matter varies but slightly within each horizon from year to year. There is, however, considerable difference between the organic matter in the leachings of the A_1 and A_2 horizons.

The differences in the organic matter from the various horizons are easily noted from the data on the C:N ratios and the nitrogen content of the organic matter. The ratio is high in A_1 and low in A_2, giving a high nitrogen content to the organic matter in A_2. This offers a clue to the observed phenomenon of a high nitrogen content in the organic matter of the B horizon.

It is pointed out that the scant leachings from B and C prevent the evaluation of the true loss of nitrogen from the soil, but even if one accepts the losses from A_2 as typical—an assumption not justified—they are small, and the addition of nitrogen by the rainwater more than offsets these losses. Appreciable amounts of nitrates are retained by the B horizon and for this reason the nitrate content in the leachings from C is, as a rule, low.

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